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ENVIRONMENT SELECTION BY DROSOPHILA MUTANTS[1]

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INTRODUCTION

In the development of the mathematical theory of evolution by Haldane, Sewall Wright, Fisher and others, the environment is normally treated as uniform and the behaviour of various mutants in respect to it is discussed simply in terms of a coefficient of selection by which each mutant is characterised. In practice it is, of course, clear that the majority of species live under conditions in which the environment is not uniform but presents many minor variations. The variant types present in the population will react with these variations of the environment in much more complicated ways than are contemplated by the simple mathematical theory. One type of interaction between animal and environment which may be expected is that different genotypes may have different preferences among the variants of environment open to them. There may then be a selection by a particular genotype of an environment in which it will be subjected to selective pressures somewhat different to those which apply to the population at large. This possibility has frequently been discussed by authors who approach evolutionary questions through a study of natural history and ecology (see review by Thorpe, 1945), who point out that different subspecies or local races may show different habitat preferences. There has, however, been little experimental study of the genetic variation in this connection within a single species not already systematically sub-divided. A small experiment has therefore been carried out on *Drosophila melanogaster* to determine whether such selection of the environment by particular genotypes can be shown to occur.

MATERIALS AND METHODS

The genotypes investigated were a number of standard laboratory stocks each characterised by the presence of one major gene. The general genetical background of the various stocks was presumably considerably diversified and the behaviour of the stock is therefore not necessarily a direct consequence of the presence of the major gene after which the stock is named. The environmental preferences of the stocks were tested in a special apparatus made of wood. This was octagonal in shape and consisted of a central chamber, approximately 11 ins. in diameter, which communicated by holes, 1 in. in diameter, with eight regularly-arranged chambers each 16 ins. long and 4 ins. across their narrower, 18 ins.

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across their broader ends. The height of the whole apparatus was 12 ins. In these eight chambers a series of environments were set up by varying the factors of temperature, light and humidity. The control of these variables was somewhat crude. During the experiments the apparatus was kept in a darkened room and light was provided by a 1000-watt bulb hanging 4 feet above the central compartment. The sectors of the apparatus which were to remain light were provided with glass lids and painted with white paint. The dark ones had wooden lids and were painted black. The dry and damp environments were obtained by putting a mass of soaked cotton wool in the damp compartments and an open Petrie dish containing calcium chloride in the dry ones. The warm compartments were electrically heated. The heating system had a thermostatic control, but owing to the low degree of thermal insulation provided by the equipment the temperature did not remain constant to closer limits than two degrees Centigrade. Each compartment contained a dish of an alcohol-acetic acid mixture to attract the flies.

The flies to be used in the experiment were counted out the day before. At the start of the experiment a number of different populations were shaken into the central compartment without etherisation. They were then left for a given length of time during which they were able to pass through the holes communicating from the central compartment with the various environmental sectors. After the lapse of the appropriate time the flies in the various sectors were etherised and counted.

Results

Some results obtained with this apparatus have been mentioned previously (Waddington, 1935b). In these first experiments the apparatus was in a room open to daylight and the control of the other environmental variables was exceedingly poor. There was therefore a considerable lack of homogeneity between successive experiments using the same stocks of flies. The results of individual experiments suggested that there were differences between various stocks in their response to light and dark, but the heterogeneity between replicate experiments made it impossible to draw any firm conclusions.

The results of more recent experiments using the somewhat improved conditions mentioned above are shown in table 1, which gives the results of three successive experiments using Wild Type, rough, aristaless, purple, apricot, black, and forked. Time allowed before counting in the respective experiments was 6 hours, 5½ hours, and 5 hours. The numbers moving consequently vary from experiment to experiment. No attempt has been made to analyse the data on mobility. Attention has been confined to the destinations of the flies that moved.

The first question is whether the differences in the distribution of the flies of any one stock in the three experiments are of an order to be expected by chance deviations from a consistent average, or whether they indicate real differences in conditions between the experiments. This can be tested by the χ^2 method. For each stock there are eight choices and three replicates, allowing the calculation of a consistency χ^2 with 14 degrees of freedom, except for the *pr* stock, where the absence of any flies in one of the chambers cuts the degrees of freedom to 12. All the stocks but one—the *ro* stock—gave a consistency χ^2 below the conventional .05 probability significance point, and the summed χ^2 for 96 degrees of freedom was 113.5, corresponding to a probability of about one in ten. Thus the triplicate experiments may be regarded as reasonably self-consistent. It is therefore legitimate to take the totals for the various stocks, as shown in the Table, and to apply the χ^2 method to them to test for differences in behaviour between the stocks. There are 42 degrees of freedom available, and the χ^2 value is 181.9, which corresponds to a

TABLE 1. *Results of triplicate experiments with seven stocks*

Stock	Expt.	Total flies	Destinations of moving flies: light dry cold	light dry hot	light wet cold	light wet hot	dark dry cold	dark dry hot	dark wet cold	dark wet hot
	1	160	7	8	11	10	2	1	4	2
+	2	200	20	13	13	17	18	3	3	4
	3	180	18	13	20	19	7	3	4	8
	Total	540	45	34	44	46	27	7	11	14
	1	170	20	7	18	4	—	1	3	1
ro	2	200	35	4	12	2	3	–	2	1
	3	200	35	9	28	12	14	3	15	1
	Total	570	90	20	58	18	17	4	20	3
	1	110	2	3	9	9	1	–	1	—
al	2	150	9	3	8	16	2	1	6	—
	3	200	10	8	18	20	2	1	—	1
	Total	460	21	14	35	45	5	2	7	1
	1	120	14	11	13	8	—	–	1	—
pr	2	200	9	8	13	13	3	1	—	—
	3	200	13	12	20	14	2	1	—	—
	Total	520	36	31	46	35	5	2	1	0
	1	100	7	7	7	4	—	–	1	—
wa	2	200	17	9	9	5	1	–	1	—
	3	200	19	21	21	11	2	1	3	3
	Total	500	43	37	37	20	3	2	5	3
	1	200	12	13	23	12	1	2	3	1
b	2	200	17	16	24	14	2	–	1	—
	3	200	15	9	40	16	6	1	4	2
	Total	600	44	38	87	42	9	3	8	3
	1	200	10	6	17	12	3	–	8	—
f	2	200	12	5	17	10	3	3	3	—
	3	200	18	5	21	16	5	4	5	10
	Total	600	40	16	55	38	11	7	16	10

probability much smaller than 10^{-20}. It may therefore be taken as proved that there are real differences in choice of destination between the flies in the various stocks.

That proves the main point, but it is of interest to look into subsidiary questions. Characterisation of behaviour differences would be much simplified if the proportion of flies choosing hot rather than cold were the same for wet and dry or for light and dark environments, and if, similarly, the choice of relative humidity or luminosity were not influenced by the other two variables. In such circumstances, the behaviour of each stock would be fully specified by three parameters, the percentages preferring respectively light to dark, dry to wet, and hot to cold. Table 2 gives the deviations between the observed totals for each stock, as shown in table 1, and the number expected on

TABLE 2. *Deviations from expected numbers on the hypothesis of independent choice criteria*

Stock	light dry cold	light dry hot	light wet cold	light wet hot	dark dry cold	dark dry hot	dark wet cold	dark wet hot
+	−1.7	−3.1	−3.5	+ 8.2	+10.7	−6.0	−5.6	+0.8
ro	+4.8	−0.7	−6.4	+ 2.3	− 3.2	−0.9	+4.8	−0.7
al	+1.6	−3.7	−5.7	+ 7.9	+ 2.5	−0.3	+1.7	−3.8
pr	−3.6	+0.4	+2.1	+ 1.1	+ 2.9	+0.3	−1.4	−1.8
wa	−2.6	+5.4	+1.7	− 4.5	− 1.0	−1.8	+1.9	+0.9
b	−9.6	+6.9	+7.2	− 4.4	+ 3.2	−0.4	−0.7	−2.1
f	+3.9	−5.0	−3.1	+ 4.2	+ 0.3	+0.8	−1.2	0.0
Total	−7.2	+0.1	−7.7	+14.9	+15.4	−8.2	−0.4	−6.7

the assumption that choice criteria are independent. Thus 45 wild type flies went into the first chamber, against an expected number of 46.7, giving a deviation of − 1.7; and similarly for the other figures. Since for each stock there are 8 observations, and the hypothesis requires four parameters—the total of flies moving, and the three preference ratios—conformity to the hypothesis for each stock can be tested by χ^2 for four degrees of freedom. Only one of the stocks, the wild type, gave a value significant at the .05 level, but the total χ^2, for 28 degrees of fredom, was 46.48, corresponding to a probability of less than .02. This is entirely due to the large χ^2 value, 14.08, for the wild type. The most just conclusion seems to be that some interaction between choice criteria is probable, though not convincingly demonstrated; but that to a first approximation the three criteria of choice can be considered as more or less independent.

Table 3 gives the analysis of choice criteria on this assumption of independence. It will be seen that there is no significant difference between stocks with respect to temperature preference, but the behaviour with respect to humidity and still more to luminosity does show very significant variation.

The results of another series of experiments with wild type and six other mutant stocks are shown in table 4. In the first two experiments, denoted L1 and L2, the central chamber was lighted, and in the other two, designated D1 and D2, it was kept dark. It is obvious at a glance that in the latter pair of trials there was a much smaller migration to the dark destination. One stock, *app*, was not represented in L1, and another, *v*, was absent in D2.

Analysis was done as before, with L1 and L2 considered as one set of duplicates and D1 and D2 as another set. In the D series, the numbers migrating to the four

TABLE 3. *Relative numbers moving according to each criterion of choice*

	+	ro	al	pr	wa	b	f	Total	X^2 6.d.f.	P
Total moving	228	230	130	156	149	234	193	1,320		
Light	169	186	115	148	137	211	149	1,115	31.392	≪.001
Dark	59	44	15	8	12	23	44	205		
Dry	113	131	42	74	84	94	74	612	20.483	<.01
Wet	115	99	88	82	65	140	119	708		
Cold	127	185	68	88	88	148	122	826	9.669	>.10
Hot	101	45	62	68	61	86	71	494		

TABLE 4. *Results of two sets of duplicate experiments with seven stocks*

Stock	Expt.	Total flies	Destinations of moving flies: light dry cold	light dry hot	light wet cold	light wet hot	dark dry cold	dark dry hot	dark wet cold	dark wet hot
+	L1	200	16	3	15	18	7	2	2	2
	L2	210	10	10	17	25	—	8	5	15
	D1	160	7	9	18	18	—	–	—	3
	D2	200	11	11	30	22	1	3	1	5
ca	L1	200	16	17	22	27	3	–	2	—
	L2	210	21	17	29	26	2	2	—	3
	D1	170	12	24	20	26	1	–	—	2
	D2	180	9	8	22	20	2	1	—	—
w	L1	180	19	8	7	13	7	3	6	5
	L2	200	18	11	7	10	4	4	8	9
	D1	200	18	20	15	26	—	1	1	9
	D2	200	5	13	15	11	6	2	8	10
e	L1	200	16	12	18	23	—	–	2	1
	L2	200	22	26	15	37	—	2	2	6
	D1	200	22	23	24	36	—	1	—	1
	D2	140	18	19	21	18	1	3	3	7
v	L1	200	16	9	22	34	11	–	8	—
	L2	150	19	8	18	5	8	2	12	9
	D1	200	24	13	34	24	1	1	1	—
stw[2]	L1	140	5	4	4	5	3	–	—	—
	L2	170	11	7	13	15	—	–	4	1
	D1	200	3	7	13	16	—	1	—	1
	D2	200	7	5	14	28	1	2	—	1
app	L2	210	21	13	17	18	4	2	4	3
	D1	120	7	8	9	11	—	1	3	—
	D2	200	7	5	8	9	1	–	4	—

dark chambers were so small as to give expectations for which χ^2 values would be suspect, so analysis was confined to the flies migrating to the four lighted chambers. In the D series, the consistency χ^2 value, for 18 degrees of freedom, was 16.58, corresponding to a probability of just above 0.5, indicating that conditions were under adequate control. For heterogeneity between stocks, the χ^2 was 34.76 for 18 degrees of freedom, significant beyond the .01 point. The D series therefore confirm the conclusion that mutant stocks differ in their choice of environment. In the L series, the consistency χ^2 was much larger than would be expected by chance, being 86.59 for 40 degrees of freedom, significant beyond the .00001 point. The discrepancies were due to the wild type and *v* flies, all the others giving low χ^2 figures. Between stocks the χ^2 was even greater, being 135.76 for 42 degrees of freedom. Even allowing for heterogeneity between duplicates, this is almost significant at the .05 level, as is shown in table 5. As far as it goes, the L series therefore also tends to support the conclusion already reached. But since the main effects are not irrefutably es-

TABLE 5. *Test of significance for L series*

	Degrees of freedom	X^2	Mean square
Between stocks	42	135.762	3.232
Between experiments	40	86.687	2.167
Ratio			1.644
.05 Point, F^{42}_{40}			1.665

tablished in the second set of experiments, it is not worth while analysing the data for secondary features.

Discussion

Although the two sets of experiments described above were of a preliminary nature, they have given definite evidence that different stocks of Drosophila exhibit definite and particular preferences when offered a choice of environment. Any attempt to study the physiological basis of the choices and the way in which they are determined by the effects of the mutant genes concerned would require much more complicated equipment and a more precise control of the conditions than were available. It is not intended to pursue such investigations in the immediate future. The experiments were intended merely to provide evidence of whether such environmental preferences actually exist or not.

Jentzer and Ludwig (1935) have recently described an experiment in which various mutant races of Drosophila were released in a rectangular box 1 metre long, one end of which was transparent and illuminated by a 40-watt lamp, while the other end was covered with dark paper. They investigated the position taken up by the flies five minutes after the beginning of the experiment. They found differences in this respect between the different stocks used, and in general came to the conclusion that those with dark body colours or dark eye colours tended to seek the light more than the others. This conclusion does not hold in our experiments, in which both dark eye colours (purple), dark body colours (black) and pale colours (apricot) all tended to shun the dark, as did aristaless, in which neither body nor eye colour are abnormal. The differences between our results and those of Jentzer and Ludwig are probably to be explained by the different experimental conditions. It is noteworthy that in their experiments the flies tended to be concentrated at the two ends of the box. This may indicate, as it appears to do at first sight, a tendency to go to the extremes of light or dark available; but perhaps the behaviour of the flies is influenced by the fact that at the ends of the box there are more solid surfaces within a given amount of space than there are in the middle. In our apparatus the structural complexity was similar in all different environments. Moreover, the difference in intensity between the light and dark holes leading out of the central compartment was probably greater than the contrast available in Jentzer and Ludwig's apparatus.

Perhaps the best previous evidence for the existence of habitat preferences among mutants of the same species is that described by Hovanitz (1948, 1953) in the butterfly *Colias eurytheme*. The females of this species exist in two forms, an orange and a white. The white phase is more active during the early part of the day, while later on the activity of the orange phase increases. The two forms are interbreeding, and co-exist in the same populations, the proportions differing according to the elevation and latitude of the territory studied. It is not clear whether the physiological difference in the activity of the two forms leads to any assortative mating or not.

It will presumably often be the case that when two forms within a population exhibit habitat preferences the selective values of the different genotypes will depend on the environment in which they spend their lives. A full discussion of the possible evolutionary consequences of this would involve much intricate mathematical theory, which the factual material available is hardly yet rich enough to justify. Certain points, however, emerge from quite simple considerations.

There are two opposite extreme cases. In the one, we may suppose that the whole population remains panmictic, and that there is free migration to the environment of choice open to all individuals. Then the fitness coefficients of the various genotypes will be composite functions of the

preferences for, and the selective values in, the different environments; and so long as these remain constant, natural selection will follow the course usual in a population with constant fitness coefficients. If, at the other extreme, mating is strictly within the group which has chosen a particular environment, and there is no migration between environments, we shall have two isolated sub-populations which will evolve each according to its appropriate fitness coefficents; if each genotype is favoured in the environment it tends to choose, selection will eventually proceed to the fixation of different genes in each sub-population. A more interesting situation arises in the intermediate case, when there is a certain amount of migration between two sub-populations in which selection is proceeding in opposite directions. Under some circumstances of this kind, the gene frequencies in one of the sub-populations might reach an equilibrium between the opposing forces of selection and migration. Moreover the equilibrium within a group will be stable provided that a chance decrease in the frequency of the favoured gene leads either to a decrease in migration or to an increase in the effectiveness of selection—a situation which is biologically not implausible.

The occurrence of habitat preferences, with different fitness coefficients in the different environments, and restricted intermingling between the sub-populations, may therefore lead to the appearance of a stable polymorphism in the population as a whole. The stability will in practice probably not be absolute, since in such a situation one would expect both the habitat preferences and the fitness coefficients to undergo gradual change; but it remains true that two forms exhibiting different habitat preferences could co-exist for a much longer time than would be possible if their ecological behaviour were the same.

In the experiments which have been recorded above there is no evidence that the various races have chosen those environmental situations in which they would be most favoured by natural selection. It may well be the case that in nature different mutants do not always choose the optimum conditions for their existence. It is, of course, only when the choice is such that it improves the chance of survival of a mutant which would otherwise be eliminated that the selection of the environment by the mutant plays an important part in evolution. It must also be remembered that the choice of habitat is made, not directly by the genotype, but by the phenotype, which has itself been influenced by environmental conditions during development. We may, therefore, be confronted by a subtle system of mutual interactions between the genotype, the effects of the environment on development, the natural selective forces and the habitat preferences of the phenotype, which can bring into play the processes of "organic selection" (Simpson 1953) or "genetic assimilation" (Waddington, 1953a).

Summary

(1) An apparatus was constructed in which a central compartment communicated with eight wedge-shaped chambers. The conditions in these chambers were rather roughly controlled so that each of them was either hot or dry, cold or wet, light or dark. Mixed groups of adult *Drosophila melanogaster* were released in a central compartment and their distribution among the various chambers investigated some hours later.

(2) In a first group of three experiments the stocks used were Wild Type and six others, each homozygous for a well-marked recessive gene; the genetic backgrounds were not standardised. When the numbers which had moved into the various compartments were analysed it was found that there was little evidence of heterogeneity between the various experiments, but overwhelming evidence of differences in behavior between the stocks. In another series, using Wild Type and

another six mutant stocks, there was also strong heterogeneity between stocks but in this case some significant heterogeneity between experiments. Although this second series is therefore not as satisfactory as the first, it provides confirmatory evidence for the existence of differences in behaviour between different mutant stocks.

(3) No attempt has been made to analyse in detail the physiological reasons for the different choices made. The facts agree to a first approximation with the hypothesis that the criteria of choice with respect to heat, light and humidity, are independent. If this hypothesis is adopted, the figures indicate that there is no significant difference between stocks with respect to temperature preferences, but that behaviour with respect to luminosity and humidity shows significant variation.

(4) It is pointed out that in a heterogeneous environment preferences of particular mutants for those environmental conditions in which they were most favoured by natural selection might under some circumstances lead to the maintenance of a relatively stable polymorphism.

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THE CORAL SNAKE "MIMIC" PROBLEM IN PANAMÁ

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The student of evolution, if his attention is called to contemporary phenomena in the Animal Kingdom, cannot fail to note cases of extraordinary superficial resemblance between animals of quite diverse relationships.

The term "mimicry" and a voluminous body of literature has resulted. In the most general terms "mimicry" may be called a word to describe a situation (or an evolutionary problem) in which external appearance, confined to a certain area and a certain size range, is shared by species of diverse ancestry. In many of these situations (and the coral snake problem is a case in point) some species are definitely venomous, poisonous, or distasteful as food while others are innocuous and edible. In all these situations the evolutionary problem of the superficial resemblance (the observational data) challenges the evolutionary student.

An almost complete acquaintance with the history, facts, and status of the coral snake mimic problem may be gained by the perusal of four papers; those of Cope 1860, Wallace 1867, Gadow 1911, and Sternfeld 1913. Cope first called attention to the existence of the problem in the following words (p. 262): "The subject of the prevalence of peculiar shades and arrangements of colors throughout certain geographical districts is one of much interest to the zoologists," "the Elaps of South America is represented in the same region by the black and red ringed Oxyrhopes, the Erythrolamprus, Pliocercus, Lampropeltis, etc." Wallace, in discussing the problem, proposed a solution: "a very remarkable and instructive case of true mimicry." Gadow, writing after extensive field experience in Mexico, objected to the idea of mimicry on a number of grounds as follows: "There are more mimickers . . . than species of *Elaps*," "there are large districts of unquestionably *Elaps*-terrain where the harmless species and individuals form the great majority." "The range of harmless species in the typical dress of *Elaps* often extends far beyond that of the nearest species of *Elaps*." "I have not yet come across a single instance of what may be called occurrence side by side." This in spite of the fact that he had previously written (1908) "several kinds of harmless snakes, belonging to different groups, inhabit the same districts together with the poisonous coral snake, and, what is more, they lead the same kind of life under rotten stumps and in ants nests."

He proposed two other solutions, as follows: (1) "at dusk . . . black, alternating with red produces an effacive blur"; i.e., the coloration of these snakes is concealing; and (2) "because the America [sic] environment favours the production of red in snakes . . . the Scientist . . . means, but does not like to say: *Genius loci*."

Sternfeld's paper is a defense of mimicry against some of Gadow's criticisms, and an attack on the second of Gadow's solutions. He suggests that superiority in the number of species of mimics over coral snakes is a point in favor of, not against, mimicry. He claims that the coincidence of range is much better than Gadow implied, and adds the important point of coincidence in size, the coral snake coloration being manifested only in snakes of coral snake dimensions. Gadow's "side by side" criticism he considers absurd, and ridicules it in the most amusing paragraph I have ever read in a scientific paper. With regard to the environment in America and the "*Genius loci*" he says, concerning this "*geheim-*

nisvolle Amerikanische etwas" which loses its power below 40 and above 100 cm., *"Ein merkwürdiger Genius."*

Since Sternfeld's paper the problem has scarcely been mentioned, and almost seems to have been shunned by common consent. Thayer (1918) considered the coloration obliterative; the rings of various colors cut up "the motionless snake's long form into short un-snake-like segments." Hingston (1933) does not discuss mimicry, and does not mention coral snakes. Cott (1940) mentions coral snakes only to say that if they are concealingly colored "then surely one might as well suggest that snails are swift and earthworms are heavily armoured," and in his section on mimicry does not mention them or their "mimics" at all.

Thus the recent books on animal coloration give scant consideration to possible mimicry of coral snakes by other snakes. The problem, however, still exists. Everyone who has dealt with these ringed snakes has noted the color similarities, and most students have been deceived at one time or another. That the markings are, under certain conditions, obliterative may be admitted (I have noted it in the field) without abandoning the additional possibility of mimicry.

Mimicry may be pictured as an evolutionary race with several contestants. First are those who assume a warning coloration to advertise their venomous nature and thus escape predators; second are those who crowd in behind the first and assume the same coloration as a disguise to delude predators; third are the predators. If the second group becomes too numerous the first group loses its advantage and further assumption of more distinctive warning coloration may be of evolutionary value. This might correspond to a sprint in a race, and might be followed up by a similar sprint on the part of the second group. There might be a second sprint, etc.

The word "numerous" in the preceding paragraph has reference to individuals only, and not at all to numbers of species or of higher categories. Gadow implies that a "great majority" of the ringed *individuals* may be harmless, and if true this would be his main point against mimicry, and this point was *not* met by Sternfeld. Gadow gave no numbers to support his statement. I cannot contradict it as far as the situation in Mexico is concerned, but it is quite contrary to the situation as I have observed it in Panamá (cf. Dunn 1949).

In Panamá, out of a total of 13,912 snakes of 108 species examined, 1175 belonged to 15 species having the "coral snake" coloration. Of the latter number 726 were coral snakes (*Micrurus*), a majority of 61.8%, as against 449 non-Micrurus, a minority of 38.2%. Of the Micrurus, 600 were *M. nigrocinctus;* of the non-*Micrurus,* 234 were *Erythrolamprus bizona.*

There were six full species of *Micrurus* and nine full species of non-*Micrurus,* representing seven genera and three subfamilies of Colubridae. Sibynophiinae: *Sibynophis venustissimus, S. zeteki,* six specimens. Xenodontinae: *Pliocercus euryzonus, Erythrolamprus bizona, E. mimus, Oxyrhopus petola,* 378 specimens. Colubrinae: *Rhinobothryum bovalli, Lampropeltis triangulum, Tantilla annulata,* 75 specimens.

Of the 1175 individuals of this color clique in Panamá the great majority (1084 individuals, 92.3%) are venomous. The species of *Micrurus* are extremely venomous; those of *Erythrolamprus, Oxyrhopus, Rhinobothryum,* and *Tantilla,* with grooved teeth on the hind end of the maxilla, are somewhat venomous; the others are not venomous.

Picado (1931) records a bite of an *Erythrolamprus bizona* on a human finger. It caused pain and swelling to the elbow, and four days elapsed before full recovery. In 1932, at El Valle, I took a *Pliocercus euryzonus dimidiatus,* and later the same day took another ringed snake, which I thought to be a *Lampropeltis,* and which

I put into the bag with the *Pliocercus*. When I opened the bag somewhat later the *Pliocercus* was dead with the jaws of the "*Lampropeltis*" (actually an *Erythrolamprus bizona*) tightly clenched on its tail.

Coral snakes can be arranged in three groups by color pattern. Some are "bicolor," with rings of red-black-red or white-black-white. This has been considered primitive. Others are "tricolor," with rings of red-yellow-black-yellow-red. Still others are "tricolor-triad," with rings of red-black-yellow-black-yellow-black-red. This has been considered the most advanced pattern.

When Panamá material is analyzed under the above three headings some interesting facts emerge.

Bicolor snakes: 184 specimens. Of these, 72 are *Micrurus* and 112 are not. The *Micrurus* are mostly *M. mipartitus* (63 specimens), but include also *M. stewarti stewarti, M. stewarti schmidti,* and adult *M. nigrocinctus yatesi.* The others are

78 *Oxyrhopus petola sebae*
13 *Pliocercus euryzonus euryzonus*
21 *Pliocercus euryzonus dimidiatus*

Tricolor snakes: 975 specimens. Of these 638 are *Micrurus* and 337 are not. The *Micrurus* are mostly *M. nigrocinctus nigrocinctus* (600 specimens), but there are also 31 *M. clarki,* and a few *M. nigrocinctus mosquitensis* and young *M. nigrocinctus yatesi.* The others are

5 *Sibynophis venustissimus*
1 *Sibynophis zeteki*
234 *Erythrolamprus bizona*
20 *Erythrolamprus mimus micrurus*
12 *Erythrolamprus mimus impar*
14 *Rhinobothryum bovalli*
12 *Lampropeltis triangulum micropholis*
37 *Lampropeltis triangulum gaigae*
2 *Tantilla annulata*

Tricolor-triad snakes: 16 specimens, all *Micrurus.* Fifteen are *M. dissoleucus dunni;* the other is *M. ancoralis jani.*

These figures are an indication that, in the Republic of Panamá as a whole, bicolor coral snakes are outnumbered by bicolor non-*Micrurus,* tricolor coral snakes are about twice as numerous as tricolor non-*Micrurus,* tricolor-triad *Micrurus* have no "mimics." These figures afford some support to mimicry as a theory to account for the colors of the "mimics," and, quite unexpectedly, to account for a progressive evolution of coral snake coloration. The theory thus acquires additional status by accounting for phenomena which were scarcely known at the time it was proposed, and for whose explanation it was not invented (cf. Davis, 1928, pp. 23–25). It is a pleasure to me to transfer the thought of my late mentor W. M. Davis, master of the evolution of land forms, from the coral reef theory of Darwin to the coral snake theory of Wallace.

The relative number of species and individuals of ringed snakes versus non-ringed snakes and of *Micrurus* versus "mimics" is naturally subject to considerable variation as one contrasts or compares one Panamanian area or locality with another. I here set down the extreme conditions, as noted in nine areas or localities from each of which more than 500 snakes have been examined. These are: upland Chiriqui, above 3500 feet (839 snakes); Finca Lérida in upland Chiriqui at 5300 feet (540); Pto. Armuelles on the Chiriqui coast (626); the lowlands of Coclé and Herrera Provinces (1350); the lowlands of the Pacific slope of the Canal Zone and the adjacent parts of the Province of Panamá, called "Sabanas" for short (4324); the lowlands of the Atlantic slope of the Canal Zone and the adjacent part of Colon Province, called "Chagres" for short (3198); the village of Agua Clara, near the Chagres River and just outside the Zone (1103); the lowlands of the Province of Darien, in the lower Tuira basin (3108); the village of Yavisa, in Darien at the mouth of the Chucunaque (2321).

The highest incidence of ringed species

in a local fauna is 10 out of 47, or 21.3%, at Agua Clara; the lowest is 3 out of 32, or 9.4%, in upland Chiriqui, and one of the three is ringed only when juvenile. Thus the *"Genius loci,"* the *"Amerikanische etwas,"* affects at most about ⅕ of the species.

The highest incidence of ringed individuals occurs in the material from the Sabanas, 499 out of 4324, or 11.5%; the lowest is in Darien, 107 out of 3108, or 3.4%. The two areas have almost identical incidence of ringed species, 18.9% and 18.2% respectively, and almost identical climates.

The species of "mimics" outnumber the species of *Micrurus* except at Lérida, which has one species of *Micrurus* and one of *Lampropeltis*. At Agua Clara only three out of ten ringed species are *Micrurus,* although 93 out of 122, or 76.2%, of the ringed individuals are *Micrurus*.

The highest incidence of *Micrurus* among ringed individuals is in the Chagres area, 269 out of 349, or 77.0%. The lowest incidence is in upland Chiriqui, 17 out of 58, or 29.3%. Here 37 of the 41 non-*Micrurus* are *Lampropeltis triangulum gaigae,* which is a "mimic" only as a juvenile, and the area is at the upper altitudinal limit of *Micrurus*. The incidence is also low in Coclé-Herrera, 24 out of 73, or 33.0%, and none of the species undergo ontogenetic color change.

A check of the series of percentages for the above relationship with those for incidence of ringed individuals in the total material from the nine areas or localities indicates quite clearly that wherever ringed individuals are relatively abundant (Sabanas 11.5%, Chagres 10.9%, Agua Clara 11.1%) *Micrurus* are in the majority (Sabanas 64.5%, Chagres 77.0%, Agua Clara 76.2%). Where ringed individuals are less frequent (Darien 3.4%, Coclé-Herrera 5.5%, Puerto Armuelles 5.3%) *Micrurus* are in the minority (Darien 47.7%, Coclé-Herrera 33.0%, Puerto Armuelles 37.9%).

Put in another way, the numerical superiority of *Micrurus* among ringed individuals decreases from a maximum in the tropical deciduous forest climate of the Chagres to a lower value in tropical savanna climate and in temperate forest in the upland Chiriqui area. It is possible that the true maximum superiority may be in tropical evergreen forest, but adequate data are lacking from such areas in Panamá. Of 21 ringed individuals from such areas 13, or 61.4%, are *Micrurus*.

In the bicolor series there is no snake that *closely* resembles *Micrurus mipartitus*. Nor is there any *close* resemblance between *Pliocercus euryzonus euryzonus* and any *Micrurus*. *Oxyrhopus petola sebae* and *Pliocercus euryzonus dimidiatus* are fairly closely similar to *Micrurus stewarti* and, to a lesser degree, adult *Micrurus nigrocinctus yatesi*.

In the tricolor series *Erythrolamprus mimus micrurus* is *identical* in color and in pattern with *Micrurus clarki*. The other "mimics" fall somewhat short of this perfection, and the head and body shape of *Rhinobothryum* (apparently arboreal) is very different from that of any *Micrurus* or any of the other ringed snakes.

Two snakes merit especial attention because of color changes during growth. *Lampropeltis triangulum gaigae* of the Chiriqui uplands lives at or above the altitudinal limit of *Micrurus*. As a small snake it could be considered a mimic of tricolor *Micrurus,* but as it grows out of the *Micrurus* size range it becomes completely black. Its lowland relative, *L. t. micropholis,* also grows out of the size range of *Micrurus,* but does not get out of the altitudinal range and undergoes no ontogenetic color change. These facts are favorable to mimicry rather than against it.

Micrurus nigrocinctus yatesi of lowland Chiriqui is, as a young snake, tricolor, and indistinguishable from its close ally *M. n. nigrocinctus*. As it grows up the red rings gradually turn black on the dorsal surface, so that a tricolor coral snake turns into a bicolor one and thus onto-

genetically reverses the suggested phylogenetic development. I have no explanation for this, and cannot consider it favorable to the theory of mimicry.

Conclusions

The difficulties of the mimicry theory are not to be minimized; they are real and great. It is to be hoped that they will be investigated and eliminated, since no other explanation of the facts seems nearly as satisfactory.

The first is the question as to whether *any* organism can distinguish colors and patterns in dim light. Notes in the literature, notes accompanying specimens, personal experience in Panamá, and retinal studies on *Micrurus* and *Lampropeltis* (cf. Walls, 1942) indicate that *Micrurus* and many if not all of the "mimics" are crepuscular if not nocturnal. This of course does not mean that none of them is ever exposed to direct sunlight. I have taken an *Erythrolamprus bizona* crossing a road in bright daylight, and the reflected rays of the sun on the polished scales of a *Micrurus n. nigrocinctus* caught my eye as from a mirror before I saw the snake or its color. Also any nocturnal, secretive snake can be exposed to daylight by the activities of a diurnal predator. But much of the activity of these snakes must take place at a time when the colors may well be invisible, and thus not subject to selection of any sort.

The second difficulty is that of the predation, which, on the mimicry theory, is the particular part of the environment responsible for evolution in the direction of warning and mimic color. Mammalian predation on snakes certainly exists, but mammals are considered to be color-blind, with the exception of Primates (Walls, 1942), and Neotropical monkeys are not notable snake predators. Avian predation on snakes exists, the most conspicuous predator on Panamanian snakes being the beautiful Ghiesbrecht's hawk (*Leucopternis ghiesbrechti costaricensis*), but it is diurnal. It, like most diurnal birds, probably has color discrimination, but nocturnal birds such as owls are considered by Walls to be achromatic, and in any case, owls are not notable predators on snakes. The requisite predators are not to be found among any group of invertebrates, nor among aquatic vertebrates. Some of the larger Panamanian frogs are capable of eating small snakes (*Leptodactylus pentadactylus* has been photographed doing so), and so are some of the larger Panamanian lizards, but they seem omni-carnivorous rather than ophiophagous. The frogs are probably, and the lizards almost surely capable of color discrimination in good light, but whether they can do so in dim light is very questionable. They do not seem to me the requisite predators.

When attention is turned to the feeding habits of the snakes themselves it immediately becomes apparent that the majority of the Panamanian individuals with coral snake color pattern are ophiophagous. *Micrurus*, in common with most of its elapine allies, is definitely addicted to eating other snakes; *Erythrolamprus* has not been reported as taking any other prey. The 922 specimens of these two genera account for 84.4% of the ringed snakes in Panama, and the *Erythrolamprus* account for 59.2% of the "mimics." *Lampropeltis* is at least somewhat ophiophagous in the United States, and if the 49 individuals of that genus be added, 88.6% of the color clique can be accounted for and 70.2% of the "mimics."

The remaining "mimic" individuals (134 specimens, 11.4% of the color clique, and 29.8% of the "mimics") are:

78 *Oxyrhopus petola*
34 *Pliocercus euryzonus*
14 *Rhinobothryum bovalli*
5 *Sibynophis venustissimus*
2 *Tantilla annulata*
1 *Sibynophis zeteki*

It may be that some of these are ophiophagous, but an investigation kindly made for me by C. M. Bogert, on American Museum specimens, indicates that the

most important one (*Oxyrhopus*) is not, and I would be surprised if any of the others turned out to be.

It is unfortunate that the great majority of the individuals seen by me have been heads-and-necks only, since one method of pursuing this problem further would be by identifying the stomach contents of these snakes to see whether ringed individuals are less subject to predation by them, and whether or not cannibalism is practiced. I have given an instance of an attack by one ringed snake on another (of a different species), but they were rather roughly forced into proximity. Picado reports what he calls a "fight" between a *Micrurus mipartitus* and a *Micrurus nigrocinctus* which "got into the cage" of the former. The *M. mipartitus* was killed, but apparently not eaten. This suggests pursuing the problem further by placing mixed lots of ringed and non-ringed snakes, *Micrurus* as well as non-*Micrurus*, together in suitable enclosures, and finding out to what extent predation is discriminatory.

In such studies it might be interesting to include *Clelia clelia*, of which 92 specimens were in the Panamanian material examined. This is a notoriously ophiophagous snake, which reaches a length of some two meters and even attacks big vipers such as *Bothrops atrox*. It is venomous. It is not a ringed snake and the adults are black, but the juveniles, in the size range of the *Micrurus* and the "mimics," are bright red. It is possible that this species is in some way involved in the color clique.

Summary

In Panamá, random sampling of the snake population indicates that a minority (38%) of the conspicuously ringed snakes are relatively or quite innocuous. These individuals represent nine species, seven genera, and three subfamilies. A majority (68%) of such snakes are very definitely venomous. These individuals represent six species, all of the same genus, *Micrurus*. These facts support the theory of mimicry.

In Panamá, a majority (some 85%) of the conspicuously ringed snakes are ophiophagous. This fact, not previously noticed, indicates some lines for further investigation.

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RETICULATE EVOLUTION IN THE APPALACHIAN ASPLENIUMS[1]

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INTRODUCTION

To be able to postulate the course of evolution in a group of organisms on the basis of indirect evidence is one of the goals of phylogenetic research, since in many groups direct evidence is difficult or impossible to obtain. Occasionally a worker may be fortunate enough to confirm his indirect and comparative techniques by direct evidence, such as the discovery of a postulated ancestral type, or the production of a postulated form by hybridization experiments. In the present study of the evolution of the Appalachian Aspleniums, a complex group of small ferns of the eastern United States, the methods used have been indirect ones, morphological, anatomical, and cytological. But in future years it is to be expected that experimental proof of the conclusions of this study will be forthcoming. The indirect steps used as evidence of reticulate evolution in the Appalachian Aspleniums are thus subject to validation by direct tests.

Wherry (1925, 1936) pointed out that the Appalachian Aspleniums "form a series showing intermediates between certain long-recognized species." His basic end-point species were five in number: *Asplenium pinnatifidum* (lobed spleenwort), *A. montanum* (mountain spleenwort), *A. bradleyi* (cliff spleenwort), *A. platyneuron* (ebony or brownstem spleenwort), and *A. rhizophyllum* (the walking-fern, usually treated as *Camptosorus rhizophyllus* but for sake of simplicity retained in *Asplenium* here). Detailed studies of the morphology of these five end-point species and comparisons of them with their various intermediates suggest that within the complex totalling 11 described entities, the real extremes are only three in number. These three species are *A. montanum, A. platyneuron,* and *A. rhizophyllum.* All the remaining taxa, commonly treated as species or hybrids—including the familiar *A. pinnatifidum* and *A. bradleyi*—lie somewhere between these three extremes in their morphology.

Among students of the Filicineae it has been rather conventional to consider as hybrids only those intermediate forms which occur as single, sterile plants with obvious parents growing nearby. But with the increasing recognition of allopolyploidy as a factor in species formation in plants, sterility *per se* is no longer the sole signpost of hybrid origin. Indeed, the best-known fern hybrid, *Asplenium ebenoides* (*A. platyneuron* × *A. rhizophyllum*), though usually sterile, occurs as a fertile form in one large population in Alabama. It has not, however, been examined cytologically until the present study (Wagner, 1953), nor have any others of the Appalachian Aspleniums been so investigated. The aim of the present work has been not only to clarify the cytological picture of the group, but also to use any other available indirect approaches to its evolutionary history. Thus certain morphological and anatomical features were found valuable in interpreting the intermediate forms. The evidence now indicates that a complex of eight described entities, *A. ebenoides, A. pinnatifidum, A. trudellii, A. kentuckiense, A. gravesii, A. bradleyi, A. bradleyi* × *A. platyneuron,* and *A. bradleyi* × *A. montanum,* has arisen as a result of hybridiza-

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tion between three ancient and original species.

The three basic species are entirely distinctive, whereas the remaining taxa constitute a difficult group, as shown by diverse herbarium identifications and taxonomic interpretations. *Asplenium montanum* (fig. 1, M) shows notably the following peculiarities: (1) triangular, long-petiolate, 2–3-times dissected leaf blades, (2) dark-brown color of the leaf axis present only at the base of the flattened petiole, (3) "glossy" upper leaf surface, resulting from the markedly elongate, practically straight-walled form of the epidermal cells, and (4) occurrence strictly in acid-rock crevices. The closest relative of *A. montanum* appears to be the wide-ranging *A. adiantum-nigrum* of the western United States, Africa, Eurasia,

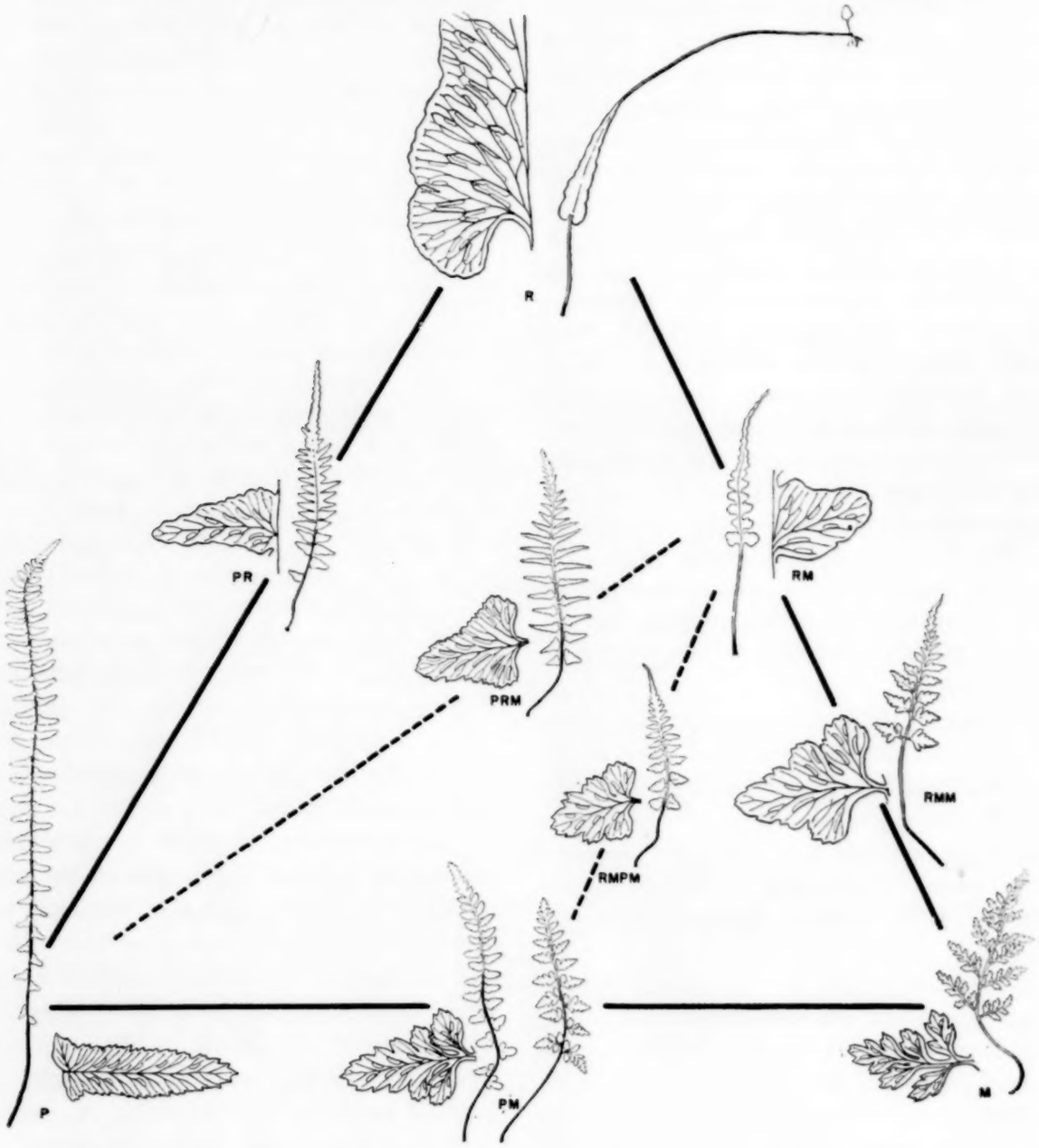

FIG. 1. Concept of relationships in the Appalachian Aspleniums. R. *Asplenium rhizophyllum*; P. *A. platyneuron*; M. *A. montanum*; PR. *A. ebenoides*; RM. *A. pinnatifidum*; PM. *A. bradleyi* (frond on left, Frederick Co., Va., *Gilbert 250*; frond on right, Madison Co., Mo., *Russell*); RMM. *A. trudellii*; RMPM. *A. gravesii*; PRM. *A. kentuckiense*.

TABLE I

Species	2n	n
Asplenium montanum		
Harford Co., Md.	72	36
Pike Co., Ohio	72	36
Asplenium platyneuron		
Montgomery Co., Md.	72	36
Harford Co., Md.	72	—
Hocking Co., Ohio	72	—
Licking Co., Ohio	—	36
Hardy Co., W. Va.	—	36
Asplenium rhizophyllum		
Ross Co., Ohio	—	36
Fairfield Co., Ohio	72	—
Shenandoah Co., Virginia	72	36
Monroe Co., Indiana	—	36
Asplenium ebenoides		
Montgomery Co., Md.	72	(72 univalents)
Hale Co., Alabama (Groff)	144	72
Hale Co., Alabama (Logue)	144	72
Asplenium pinnatifidum		
Shenandoah Co., Va.	144	72
Hardy Co. W. Va.	144	72
York Co., Pa.	—	72
Licking Co., Ohio	—	72
Monroe Co., Indiana	—	72
Asplenium bradleyi		
Harford Co., Md.	144	72
Pike Co., Ohio	144	—
Asplenium trudellii		
York Co., Pa.	108	(ca. 36 univalents, ca. 36 bivalents)
Hardy Co., W. Va.	108	(ca. 36 univalents, ca. 36 bivalents)
Pike Co., Ohio	108	(ca. 36 univalents, ca. 36 bivalents)

(Voucher specimens deposited in the Department of Botany University of Michigan)

and Hawaii, a species which also possesses the "glossy" upper epidermal cells. *A. montanum* is confined to the eastern United States, occurring from northern Georgia and Tennessee in the Appalachian Region to western Massachusetts. *A. platyneuron* (fig. 1, P), the second of the basic species, has the following distinctions: (1) linear- to oblanceolate-elliptic, once-pinnate, short-petiolate leaf blades, (2) leaf axis entirely dark-brown, including the rounded midrib, (3) upper epidermal cells oblong, undulate-walled, and (4) occurrence on rocks and soils of a variety of pH reactions. It appears to be most closely related to *A. trichomanes* and *A. resiliens*, and its hybrids with the former have been twice recorded (*A. virginicum* Maxon). *A. platyneuron* has a very wide range, extending from Texas and Florida in the south to Wisconsin and Kansas to southern Quebec in the north. *A. rhizophyllum* (*Camptosorus rhizophyllus*) possesses these character-

istics: (1) triangular-attenuate, simple leaf-blades, with extremely long, rooting tips, (2) leaf axis green except for the base of the petiole, (3) upper epidermal cells as in *A. platyneuron,* but the venation pattern anastomosing, and (4) occurrence confined almost exclusively to well-shaded, moss-covered tops and sides of rocks and boulders, the pH reaction of the rock substratum predominantly circumneutral. Its closest relative is *A. sibiricum* of northeastern Asia. *A. rhizophyllum,* like *A. platyneuron,* is a rather common fern and has a broad range, extending from Alabama and Georgia north to Minnesota and Quebec. The morphological characteristics of these three basic species are intricately blended among the members of the Appalachian Asplenium complex. The chromosome numbers (table 1) of the basic species cannot be used to distinguish them since they all have the same —$2n = 72$, with 36 pairs at meiosis. The number 36 seems to be characteristic of the entire family Aspleniaceae, as was found in European species of *Asplenium, Ceterach,* and *Phyllitis* by Manton (1950) and Hawaiian species of *Diellia* by Wagner (1952).

Methods

Observations upon the different described taxa were made in the field and in the herbarium. Some of the entities are, however, exceedingly rare, and field observation of these was impossible, although localities where they had been collected in the past were visited. Dried leaflets were cleared in sodium hydroxide solutions, stained with tannic-acid and iron chloride, and mounted on microscope slides in diaphane in order to examine venation patterns and other anatomical features. Leaf-outline drawings were made on a tracing table, and epidermal-cell drawings with a Bausch and Lomb microprojector. For the cytological study, living plants were grown under greenhouse conditions by Mr. Walter F. Kleinschmidt at the University of Michigan Botanical Gardens. Somatic chromosome counts were obtained from crosiers and young leaflets placed in saturated aqueous solution of paradichlorobenzene for 3 hours to shrink the chromosomes, then placed in fixative. Meiotic studies were made from young sori, fixed directly. The fixative used was 4 parts chloroform; 3 parts ethyl alcohol; and 1 part glacial acetic acid. The specimens were squashed after 24 hours in the solution, and stained in aceto-orcein.

Asplenium Ebenoides

The first of the intermediates to be discussed is *Asplenium ebenoides.* Probably no other fern of the New World has attracted so much attention as this hybrid. Wherever its parents, *A. platyneuron* and *A. rhizophyllum,* grow in close proximity, this intermediate appears with gratifying regularity, but usually as a solitary plant, and with sufficient rarity to make its discovery a challenge to the plant collector. Although its hybrid origin was strongly suspected, the finding of the large and obviously self-reproducing population of this fern at Havana Glen, Hale Co., Alabama, some eighty years ago, cast doubt on its true nature. Nevertheless, the careful experiment of Slosson (1902) produced plants by hybridization of the putative parents which were morphologically like *A. ebenoides,* thus firmly establishing the hypothesis of its hybrid origin. Until the present, however, the cytological behavior of the hybrid remained unknown, and the capacity of the Alabama population to reproduce remained unexplained. Two possible hypotheses—hereditary obligate apogamy, and allopolyploidy—were considered as possible explanations of the fertility of this usually sterile plant. Obligate apogamy, it should be noted, is already known in two species of *Asplenium, A. monanthes* and *A. resiliens,* both of which, though chiefly subtropical, occur in the eastern United States.

The cytological studies of two living plants of the usual, sterile form of *A. ebenoides* from Montgomery Co., Maryland, reveal that there is complete non-

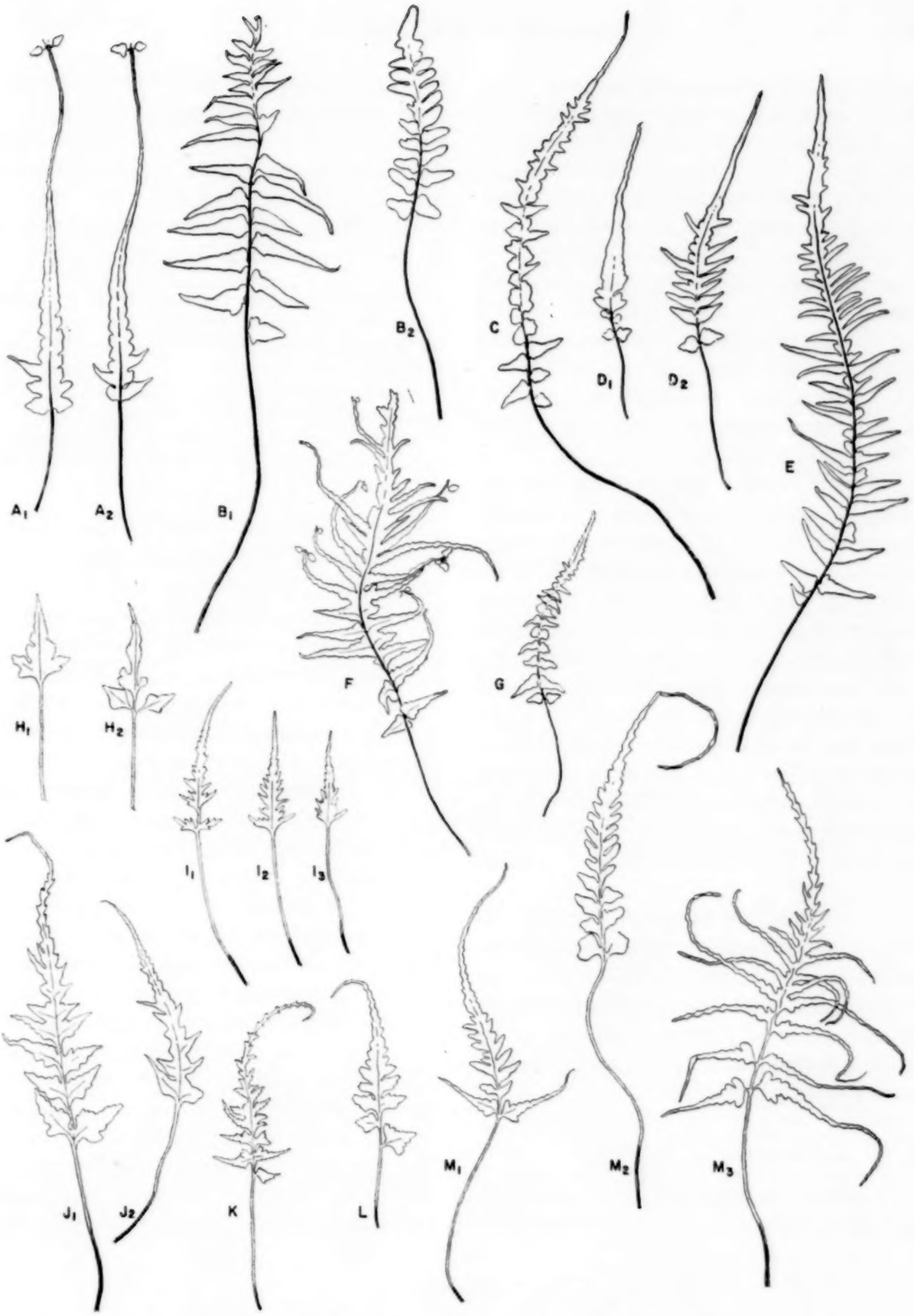

FIG. 2. Leaves showing extreme irregularity. A-G. *Asplenium ebenoides* (*Asplenium rhizophyllum* × *A. platyneuron*): A. Jefferson Co., W. Va., *Wagner & Rawlings 2016*; B. Montgomery Co., Md., *Palmer 1899*; C. Shenandoah Co., Va., *Wagner 241*; D. Hale Co., Ala., *Maxon & Pollard*; E. Hale Co., Ala. (cultivated, *Groff*); F. Loudoun Co., Va., *Gilbert 237*; G. Montgomery Co., Md., *Wagner*. H. *Asplenium inexpectatum* (*A. rhizophyllum* × *A. ruta-muraria*), Adams Co., Ohio, *Braun*. I-M. *Asplenium pinnatifidum* (*A. rhizophyllum* × *A. montanum*): I. Patrick Co., Va., *Heller*; J. Jefferson Co., W. Va., *Palmer*; K. Jackson Co., Ill., *French*; L. Madison Co., Mo., *Pinkerton*; M. Warren Co., Ky., *Sadie Price*.

pairing of chromosomes in meiosis. At meiotic metaphase there are visible 72 univalents, 36 from each of the parents (fig. 7, C). By contrast, the investigations of fertile Alabama specimens of this taxon from two collections reveal that the allopolyploid hypothesis explains the situation: dividing somatic cells show 144 chromosomes (fig. 7, D_1), and during the first meiotic metaphase 72 normal-appearing chromosome pairs are evident (fig. 7, D_2). The spores produced are normal, but like the stomata, are larger than those of the diploid relatives.

The only certain record of the normal, sexual life cycle in *A. ebenoides* is this occurrence in Hale Co., Alabama. Whether vegetative reproduction explains the multiple plants sometimes found in other localities is not known. *A. ebenoides* may produce young plants not only at the tips of the leaf blades, but also at the tips of the pinnae; as many as 8 or 10 young plants may be produced on a single luxuriant frond. The large fronds of this plant are usually conspicuously irregular in the lobulation of the blade (fig. 2, A-G), giving them a characteristic bizarre appearance.

It is possible that the origin of the fertile form of *A. ebenoides* by allopolyploidy occurred in recent times and only in one locality, and that this explains why its distribution is so narrow compared to the broader distributions of the two following intermediate types.

Asplenium Bradleyi

"Bradley's spleenwort" or "cliff spleenwort," though wide-ranging from Oklahoma and Georgia to New Jersey, is rather rare, becoming abundant only locally on steep rock cliffs. However, in recent times the possibility of hybrid origin of this fern has received no attention. As early as 1880, D. C. Eaton, who described it as a species, wrote the following:

> "*A. bradleyi* varies a good deal in the shape of the fronds and in the degree of incision of the pinnae, the narrower and less divided forms having some resemblance to *A. platyneuron* and the larger forms looking more like *A. montanum*, or the European *A. lanceolatum*. If there could be a hybrid between *A. platyneuron* and *A. montanum*, it would be very much like our plant."

Figure 1, PM, shows two of the different leaf forms of this taxon to illustrate its variability. With present-day knowledge of hybridization in the ferns, and with the evidence now at hand, its describer would not have expressed as much doubt as to its possible hybrid nature. Morphologically it is indeed intermediate between the two parents suggested by him: The rachis of *A. platyneuron* is dark brown, and nearly round in cross-section (fig. 3, P), with two delicate ridges running along the top side, the ridges separated from each other by a distance of approximately one-half the diameter of the rachis. In contrast, *A. montanum* has a green rachis which is flattened-triangular in cross-section, the two adaxial ridges appearing as conspicuous, rounded flanges, separated by a wide, shallow groove containing a medial bulge. The midrib of *A. bradleyi* (fig. 3, PM) is intermediate, being brown only in the lower half, and but slightly flattened; the two ridges are separated from each other a distance approximately

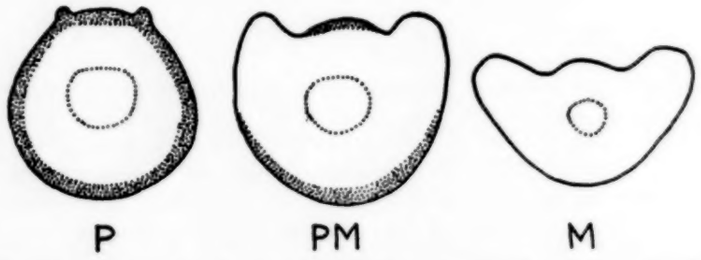

Fig. 3. Diagrams from tracings of midrib cross-sections: P. *A. platyneuron;* PM. *A. bradleyi;* M. *A. montanum.*

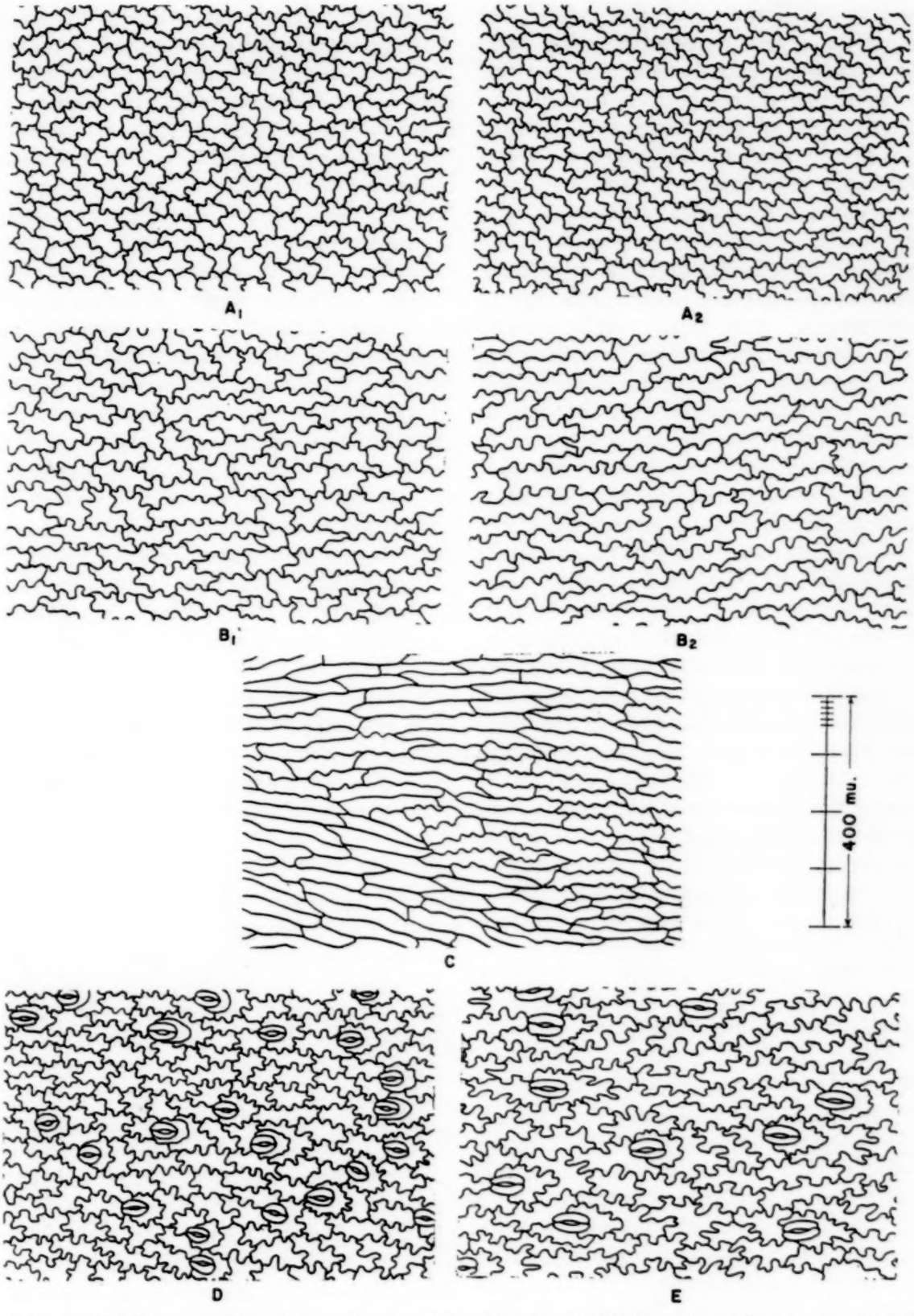

FIG. 4. Epidermal cells. A-C. Upper epidermis: A. *Asplenium platyneuron:* A_1. Duval Co., Fla., *Churchill;* A_2. Bullock Co., Ga., *Harper.* B. *A. bradleyi:* B_1. Dade Co., Mo., *Steyermark 40273;* B_2. Etowah Co., Alabama, *Eggert.* C. *A. montanum,* Alleghany Co., N. C., *Correll 10817.* D, E. Lower epidermis: D. *A. platyneuron,* Duval Co., Fla., *Churchill;* E. *A. bradleyi,* Dade Co., Mo., *Steyermark 40273.*

equal to the diameter of the rachis, and the upper groove formed between them shows only a slightly elevated, central bulge. Such other *A. platyneuron* features as more or less truncate basal margins of the leaflets and reduced lower pinnae are combined with the elongated tendency of the upper epidermal cells (fig. 4, cf. A, B, and C), the stalked and divided basal pinnae, and the restriction to acid-rock habitats characterizing *A. montanum.*[2]

No individuals of *A. bradleyi* have yet been recorded which are diploid and sterile, although they should be sought for in habitats where the supposed parents occur side-by-side in appropriate conditions. The chromosome number of *A. bradleyi* populations which have been studied is in accord with the allopolyploid hypothesis, being 144, with normal pairing at meiosis (fig. 7, F). Hybrids of *A. bradleyi* have been recorded with both *A. platyneuron* and *A. montanum*. Probably *A. bradleyi* originated by allopolyploidy in the past at least once, and perhaps several times, but it now has a rather broad range, extending in particular well to the west of the westernmost known limit of one of its putative parents, *A. montanum*.

Asplenium pinnatifidum

This intermediate is a familiar but rather uncommon fern which occurs from Alabama and Georgia northward to New Jersey, Indiana, and Oklahoma. Its resemblance to *A. ebenoides* is sufficiently close so that the two species are frequently confused. Eaton wrote (1879) that it "bears considerable resemblance to *A. ebenoides* but has a green, herbaceous midrib or rachis, a sinuous-margined prolongation, thicker texture, and is very rarely, if indeed ever, proliferous." Like *A. ebenoides, A. pinnatifidum* frequently attracts attention by the irregular appearance of its leaf-forms (e.g., Copeland 1902), to be discussed below. The larger fronds in any population may be expected to have elongated pinnae at the base of the blade (forma *elongatum* Morton). *A. pinnatifidum* also resembles *A. ebenoides* in combining the peculiar features of *A. rhizophyllum* with those of the more typical Aspleniums. Concerning the genus *Camptosorus* as conventionally recognized and typified by *A. rhizophyllum,* Copeland (1947, p. 170) wrote: "*Camptosorus* is evidently derived from *Asplenium*. *A. pinnatifidum* Nutt. presents a more definite place of origin in the parent genus." Thus Copeland considers *A. pinnatifidum* (which has also been treated as *Camptosorus pinnatifidus* Wood) as an *Asplenium* prototype of *Camptosorus*. Perhaps the most suggestive resemblance of the two taxa is the attenuated, simple tip of the leaf which, though usually shorter in *A. pinnatifidum* is much like that of *A. rhizophyllum*. However, production of young plants on the leaf tip in *A. pinnatifidum* is decidedly rare and I have found only a few examples. The other major distinction of *A. rhizophyllum* from more typical Aspleniums, that of anastomosing veins, is much more common than bud-production in *A. pinnatifidum,* but the vein areoles form casually and infrequently.

The likelihood is far greater that the *A. rhizophyllum*-like features of *A. pinnatifidum,* rather than indicating community of origin, arose abruptly through hybridization of *A. montanum* with *A. rhizophyllum*. The first suggestion in the literature of this possibility seems to be that by the writer (1950). The chromosome numbers of the five populations thus far investigated accord with this hypothesis, having the 4*n* number, and four additional populations examined showed the corresponding large-sized stomata. Not only does *A. pinnatifidum* show tendencies toward the features of anastomosing veins, attenuate leaf-tip, and leaf-tip reproduction of *A. rhizophyllum,* but its lamina is thick-textured as in *A. montanum;* moreover there is a definite tend-

[2] Reports of *A. bradleyi* on "limestone" have proved to be erroneous. Cf. Wherry, Amer. Fern Jour., **21**: 111, 1931.

ency toward elongation of the upper epidermal cells, and the basal pinnae or lobes, though simple, have the same outline as in *A. montanum*. The habitat of *A. pinnatifidum* is usually mediacid or subacid soil on rock cliffs as in *A. montanum*, although Craw (1932) found 3 occurrences in Indiana to have neutral soil-reaction, and the writer has seen it in close association with *Pellaea glabella*, a predominantly calcareous soil plant, at Black Hand, Ohio.[3]

One distinctive feature of *A. pinnatifidum* which points especially strongly to its hybrid origin is the irregularity of the blade outline, seen primarily in larger leaves. The two other hybrids between *A. rhizophyllum* and more typical Aspleniums (*A. platyneuron*, fig. 2, A-G; *A. ruta-muraria*, discovered by Braun, 1939, fig. 2, H) likewise display this condition. Rather than a symmetrical system of pinnae or lobes as is characteristic of most ferns, the blades of these hybrids show conspicuous irregularities in pattern, short lobes next to long ones on the same side of the blade, and different patterns of lobulation on opposite sides.[4]

Asplenium trudellii

Oddly enough, the widespread plant known as *A. trudellii*, which has been interpreted both as a variant of *A. pinnatifidum* and as its hybrid with *A. montanum*, lacks the peculiarity of irregular leaf-blades, a feature which is sometimes helpful in distinguishing it from *A. pinnatifidum*. *A. trudellii* has been investigated cytologically from three localities, respectively in Pennsylvania, West Virginia, and Ohio, and the findings not only bear on its own interpretation but on that of *A. pinnatifidum* as well. Although its describer considered it "possibly in part the result of hybridization between *A. pinnatifidum* and *A. montanum*" (Wherry, 1925), its most recent treatment (Morton, *in* Gleason, 1952) is as a variety of *A. pinnatifidum*. In 1932, however, Dr. Paul Kestner of Lausanne, Switzerland, found that spores of all specimens of *A. trudellii* he received from Georgia and Tennessee were sterile. In spite of the sterility of its spores—which may also be recognized under the microscope by their irregularity and abortion—this plant, remarkably, is often relatively common where it occurs. But apparently it is never found in the absence of the two presumed parents, *A. pinnatifidum* and *A. montanum*.

Present cytological evidence makes it probable that *A. trudellii* is a hybrid, but its morphology, though intermediate, usually seems somewhat closer to *A. pinnatifidum* than to *A. montanum*. The chromosome number in all the plants from the three populations studied is 108, i.e., 3*n*. Also at meiosis there are approximately 36 pairs and 36 univalents, as would be expected in a backcross of an allopolyploid hybrid of *A. montanum* and *A. rhizophyllum* with one of its parents. In 18 well-spread sporocytes the total units of any kind estimated averaged 73.2 (70–75), with 36.3 bivalents (33–42) and 36.9 univalents (33–39). The genetic constitution of *A. trudellii* may thus be now considered to be two genomes of *A. montanum* and one genome of *A. rhizophyllum*.

In spite of its production in considerable numbers, it is dubious whether *A. trudellii* will ever become a reproductive species like *A. pinnatifidum*, because its triploid chromosome complement makes this unlikely. *A. pinnatifidum*, on the other hand, is reproductively a normal species in its present-day behavior, with a typical, sexual life cycle. Its rather

[3] Dr. E. T. Wherry has since informed me that the same species-association occurs at Cumberland Falls, Kentucky.

[4] The phenomenon of irregularity of leaves of putative hybrids between ferns with widely different leaves is not confined to the Aspleniaceae; it appears, for example, in the Aspidiaceae, *Pleuroderris michleriana* (*Tectaria incisa* × *Dictyoxiphium panamense*), and in the Pteridaceae, *Pteris heteromorpha* (*P. cretica* × *P. vittata*) and *P. cadieri* (*P. cretica* × *P. quadriaurita*).

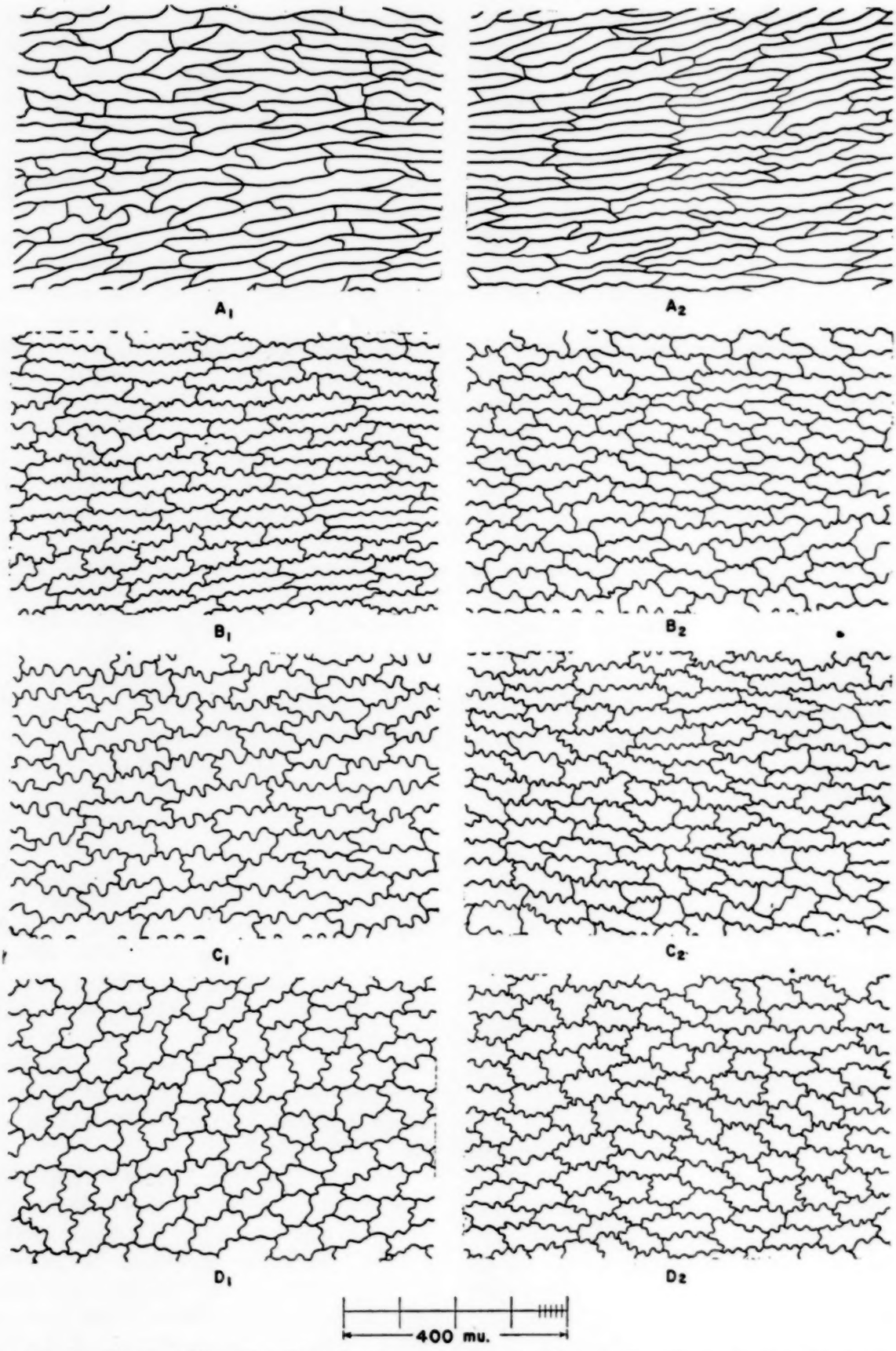

FIG. 5. Upper epidermal cells. A. *A. montanum:* A_1. Macon Co., N. C., *Correll 6679;* A_2 Sevier Co., Tenn., *Tryon 33.* B. *A. trudellii:* B_1 Etowah Co., Alabama, *Eggert;* B_2. Kentucky, *Rule.* C. *A. pinnatifidum:* C_1. Carter Co., Mo., *Steyermark 11875;* C_2. Union Co., Ill., *Hubricht B2287.* D. *A. rhizophyllum:* D_1 Smith Co., Va., *Small;* D_2. Shenandoah Co., Va., *Artz 900.*

wide range suggests ancient origin at least once by doubling of chromosomes in an original hybrid. Plant collectors should certainly seek in the field individuals of *A. pinnatifidum* which are diploid and sterile, to be expected where *A. rhizophyllum* and *A. montanum* occur in close proximity.

Asplenium kentuckiense and A. gravesii

Asplenium kentuckiense, discovered and described first by McCoy (1936), represents the theoretical central point morphologically in the entire Appalachian Asplenium complex. Although McCoy did not discuss its hybrid origin, Wherry (1936) first suggested that it represented an intermediate between *A. pinnatifidum* and *A. platyneuron.* (The other plant, *A. stotleri,* mentioned by Wherry in this connection, is now believed to involve an additional species outside the complex.) Besides the morphology of *A. kentuckiense,* which is intermediate between the two supposed parents, additional observations indicate that it is the cross of *A. platyneuron* and *A. pinnatifidum:* (1) three plants were collected by Mr. Floyd Bartley in Hay Hollow, Pike Co., Ohio, in the immediate vicinity of the two putative parents; (2) in southern Illinois, where *A. kentuckiense* was found by Earl (specimen in Chicago Museum), only *A. platyneuron* and *A. pinnatifidum* of possible parents are present, neither *A. bradleyi* nor *A. montanum* being known from that state. Specimens believed to represent *A. platyneuron* × *A. pinnatifidum* are now known from scattered points in Kentucky, Ohio, Illinois, and Arkansas, and they all possess abortive spores. Although no living plants have been available for study, the stomatal sizes of preserved material indicate the 3*n* condition.

Stomatal size seems especially useful in the determination of dried material of the rarer ferns in this complex, the material being relaxed in sodium hydroxide solutions, cleared, and treated as described above. The stomata of one pinna or fragment of all these plants under discussion may vary in length as much as 11 to 18 microns, and the averages of different collections of one species as much as 5 to 10 microns. Nevertheless it has usually proven possible by averaging 30 measurements of stomatal lengths of a pinna from a single collection to distinguish the polyploid levels. The fertile tetraploids have conspicuously large stomata. Individual collections of known or theoretical triploids may have values which overlap some of the diploids and some of the tetraploids, as shown in the chart below of averages of 30 stomatal lengths in microns of each of 31 collections:

Diploids

(range of averages of 11 collections, 39–45).
A. rhizophyllum (3 collections)..41 (40–41)
A. ebenoides (2 collections).....40 (39–40)
A. platyneuron (3 collections)...42 (40–43)
A. montanum (3 collections).....42 (40–45)

Triploids

(range of averages of 9 collections, 42–51).
A. bradleyi × *montanum* (2 collections) 44 (42–45)
A. trudellii (4 collections).....46 (42–51)
A. kentuckiense (3 collections)..45 (43–48)

Tetraploids

(range of averages of 11 collections, 46–58).
A. gravesii (4 collections).....49 (46–53)
A. ebenoides (1 collection).....54
A. pinnatifidum (3 collections)...55 (50–58)
A. bradleyi (3 collections).....56 (52–58)

Asplenium gravesii, described by Maxon (1918) as a hybrid of *A. pinnatifidum* and *A. bradleyi,* has been found in a limited number of localities in Georgia, Alabama, Ohio, and Pennsylvania. "Judging *A. gravesii* on both gross and minute characters, there can be little doubt of its hybrid nature, making all allowance for the unusually high variability of the supposed parents, of one or the other of which it might at first be thought an extreme state" (Maxon, loc. cit.). *A. gravesii* may be distinguished from *A. kentuckiense* by having less brown on the leaf-axis, pinna shape, thicker texture of the blade, somewhat more elongate upper

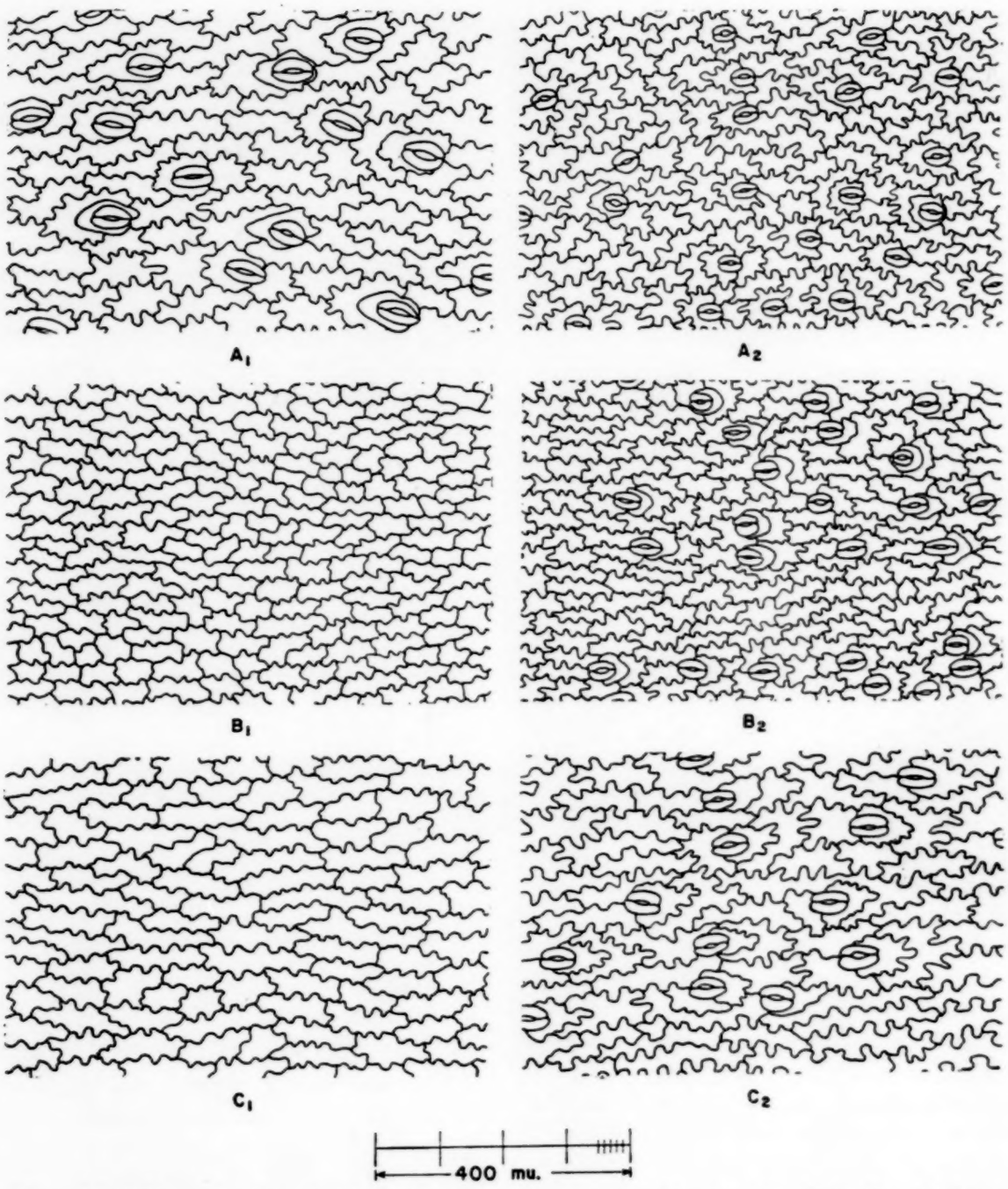

FIG. 6. Epidermal cells: A. *Asplenium ebenoides,* upper epidermis: A_1. Hale Co., Alabama, *Maxon & Pollard* (4*n*); A_2. Montgomery Co., Md., *Wagner.* B. *A. kentuckiense,* S. Illinois, *Earl:* B_1. Upper surface; B_2. Lower surface. C. *A. gravesii,* Trenton, Ga., *Graves:* C_1. Upper; C_2. Lower surface.

epidermal cells (cf. fig. 6, B and C), and the usually somewhat larger stomatal sizes suggesting the 4*n* state. However, the two ferns are similar morphologically, and young plants especially may prove to be difficult to identify on gross characteristics. Both are exceedingly rare, and no living material has been seen by the author or is expected in the near future.

These two intermediate ferns, *A. gravesii* and *A. kentuckiense,* combine morphologically distinctions of all three of the basic diploid species, in respect to such features as the extent of brown on the leaf-axis, the degree of attenuation of the leaf-tip, pinna shape, etc. But *A. kentuckiense* possesses unusual theoretical interest in this problem since it presumably repre-

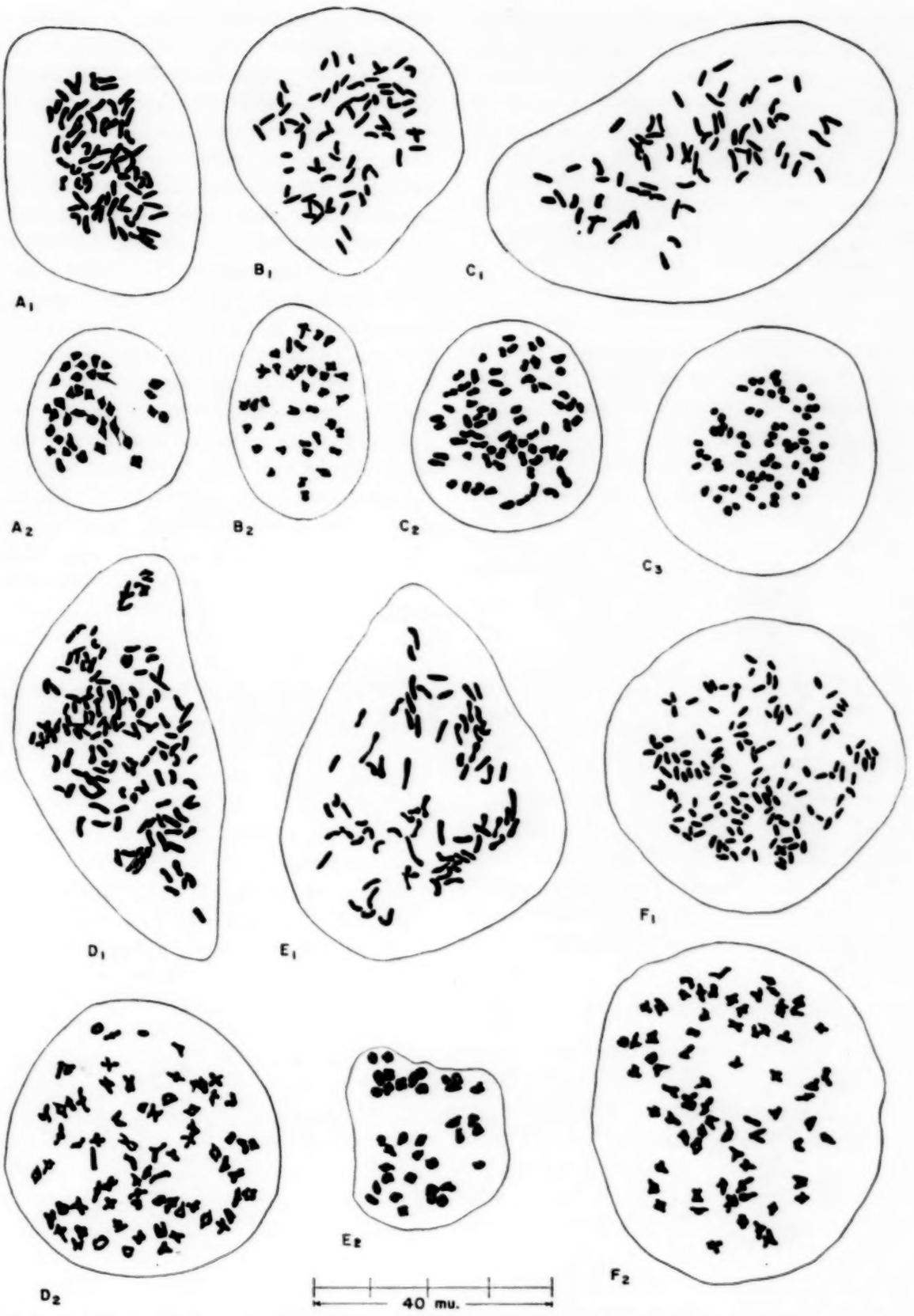

FIG. 7. Chromosomes of Appalachian Aspleniums. A. *Asplenium platyneuron:* A_1. Montgomery Co., Md., $2n = 72$; A_2. Hardy Co., W. Va., 36 pairs. B. *Asplenium rhizophyllum:* B_1. Shenandoah Co., Va., $2n = 72$; Monroe Co., Indiana, 36 pairs. C. *Asplenium ebenoides* (sterile form), Montgomery Co., Md.: C_1. $2n = 72$; C_2 and C_3. 72 univalents at meiotic metaphase. D. *Asplenium ebenoides* (fertile form), Hale Co., Alabama: D_1. $2n = 144$; D_2. 72 pairs. E. *Asplenium montanum*, Harford Co., Md.: E_1. $2n = 72$; E_2. 36 pairs. F. *Asplenium bradleyi:* F_1. Pike Co., Ohio, $2n = 144$; F_2. Harford Co., Md., 72 pairs.

sents the precise morphological central point between the "poles" of the triangle of basic, diploid species (fig. 1, PRM). Thus it should theoretically be possible to produce triploid *A. kentuckiense* in all of the following experimental crossings:

A. montanum (2*n*) × *A. ebenoides* (4*n*)
A. rhizophyllum (2*n*) × *A. bradleyi* (4*n*)
A. platyneuron (2*n*) × *A. pinnatifidum* (4*n*)

Discussion

Stebbins (1950) has discussed the role of polyploidy in plant evolution, and cites a number of established or suspected allopolyploids among flowering-plants, including *Galeopsis tetrahit* (*G. pubescens-speciosa*) and *Iris versicolor* (*I. setosa-virginica*) and others which occur as normal species. Only recently, however, has allopolyploidy in species of the Filicineae been subjected to investigation: Manton (1950) has shown that seven European ferns with normal life-cycles, including *Dryopteris filix-mas* and *Polystichum aculeatum,* are either suspected or demonstrated allopolyploids. More recently (Manton and Walker, 1953) it has been shown that the American *Dryopteris clintoniana* is a hexaploid and "is perhaps the amphidiploid hybrid between normal *D. cristata* [a tetraploid species] and *D. goldiana* [a diploid]."

Perhaps the most interesting aspect of the Appalachian Asplenium complex is that the polyploid taxa combine features

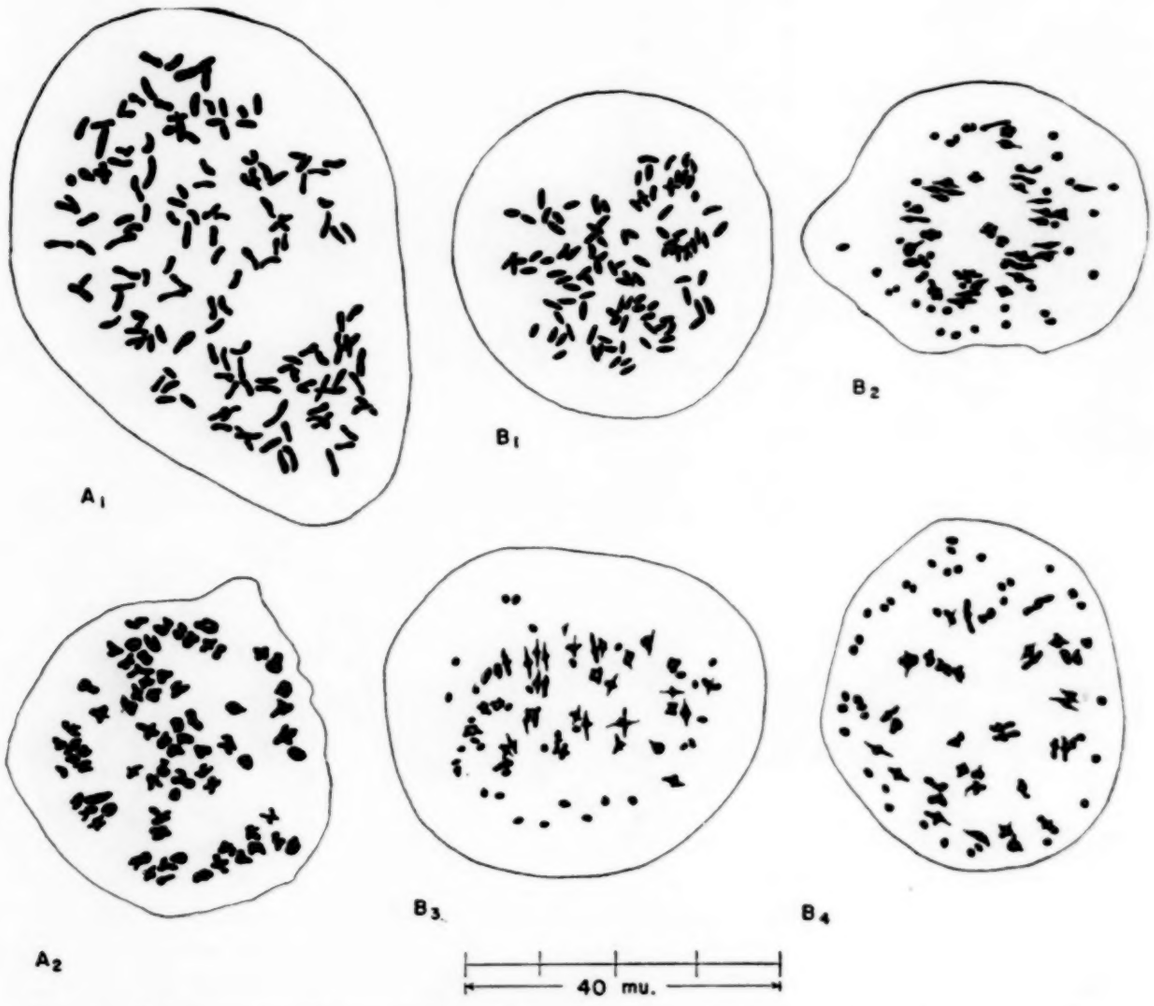

Fig. 8. Chromosomes of Appalachian Aspleniums. A. *Asplenium pinnatifidum:* A_1. Shenandoah Co., Va., $2n = 144$; A_2. Monroe Co., Indiana, 72 pairs. B. *A. trudellii:* B_1. York Co., Pa., $2n = 108$; B_2. York Co., Pa., approximately 36 pairs and 36 univalents at meiotic metaphase; B_3, B_4. Hardy Co., W. Va., approximately 36 pairs and 36 univalents.

of diploids of widely different morphology and relationship. The suspected allopolyploid, *"Scolopendrium hybridum,"* of Europe (Manton, 1950) likewise combines features of very unlike putative parents, *Asplenium* (*Scolopendrium*) *hemionitis* and *A.* (*Ceterach*) *ceterach*. The polyploids of the Appalachian Asplenium complex appear to be "typical or genomic allopolyploids" (Stebbins, op. cit., p. 226) resulting from hybridization of distantly related species in which the chromosomes are so different that normal pairing would be impossible in the diploid. The polyploid intermediates between the presumably basic diploid species in this complex are clear-cut intermediates, and there is evidently no tendency toward introgression between parent species since gene interchange through the diploid hybrids is extremely unlikely.

Two of the diploid species of this complex, *Asplenium rhizophyllum* and *A. platyneuron,* are much more abundant than their apparent allopolyploid derivatives, in contrast to many of the previously reported allopolyploids among plant species. One of the allopolyploids discussed here, namely *A. ebenoides,* is very limited in range, while the two others, *A. bradleyi* and *A. pinnatifidum*—although generally less common than *A. montanum* where their ranges overlap with it—have extended their ranges farther westward than this presumably parental, diploid species, but not farther northward.

Of the theoretically possible sterile hybrids in this complex, five still remain to be discovered, but their arising under natural field conditions seems extremely unlikely because of parental differences in ranges and habitat preferences. Even what is perhaps the most expected of the still unknown hybrids, *A. rhizophyllum* × *A. pinnatifidum,* may never be found under natural conditions because these species so rarely occur close together. Of the four theoretically possible fertile polyploid hybrids, only one—hexaploid *A. kentuckiense*—has not yet been found in the natural state, although the possibility of the origin of such a form should be recognized by field-workers.

Conclusions

The Appalachian Aspleniums comprise 11 described taxa, of which 3 represent morphological extremes and the remainder intermediates. *A. ebenoides* is a usually sterile hybrid of *A. platyneuron* and *A. rhizophyllum* with 72 univalents at meiotic metaphase, although one population from Alabama is a fertile allopolyploid, forming 72 normal bivalents at meiosis. *A. bradleyi* is apparently the allopolyploid hybrid of *A. montanum* and *A. platyneuron;* it back-crosses with both parents. *A. pinnatifidum* likewise is evidently an allopolyploid hybrid, and its morphology, the irregularity of the leaves, and the pairing behavior of its putative backcross (*A. trudellii*) with one of the parents indicates that its parentage is *A. montanum* × *A. rhizophyllum*. *A. kentuckiense* and *A. gravesii* are both evidently trihybrids, the former 3*n*, the latter 4*n*, and *A. kentuckiense* morphologically represents the theoretical central point of the whole complex. A hypothesis of reticulate evolution is thus presented for the Appalachian Aspleniums which postulates that three original diploid species, *A. montanum*, *A. platyneuron*, and *A. rhizophyllum*, have given rise to 8 additional taxa through hybridization.

Acknowledgments

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U. S. National Museum, Chicago Museum, University of Illinois, and Missouri Botanical Garden. Mr. Hubert M. Vogelman helped me with the drawings.

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A FURTHER STUDY OF CHROMOSOMAL POLYMORPHISM IN *DROSOPHILA WILLISTONI* IN ITS RELATION TO THE ENVIRONMENT[1]

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The Working Hypothesis

Natural populations of many species of Drosophila are mixtures of chromosomal types which differ in inversions of blocks of genes. This polymorphism is known to be in the main adaptive, and most of the chromosomal variants are maintained in the populations owing to a superiority of heterozygotes over homozygotes (heterosis). The amount of polymorphism is, however, variable from species to species, as well as in populations of the same species. Some species are chromosomally uniform, while in *Drosophila willistoni, D. paulistorum,* and *D. subobscura* about 40 different gene arangements per species are known (Patterson and Stone, 1952; Dobzhansky, 1951; Mainx, Koske, and Smital, 1953). In some species and populations inversion heterozygotes are rare or absent, while in populations of *D. willistoni* which inhabit central Goyaz (Brazil) the average number of heterozygous inversions per individual is about 9, and one individual heterozygous for 16 inversions has been encountered (da Cunha, Burla, and Dobzhansky, 1950).

The reasons for these spectacular variations in the degree of polymorphism are a challenging problem. Da Cunha, Burla, and Dobzhansky (1950) and Dobzhansky, Burla, and da Cunha (1950) have advanced a working hypothesis, according to which the amount of adaptive polymorphism carried in a population is, other things being equal, a function of the variety of biotic situations, or ecological niches, which the population exploits. Indeed, many more kinds of gene arrangements occur in the common and widespread species *D. willistoni* and *D. paulistorum* than in the less widely distributed, local, and usually rare, but otherwise very closely related, *D. tropicalis* and *D. equinoxialis.* In *D. willistoni* highly polymorphic populations occur near the center of the distribution area of the species, in territories with diversified floras which yield varied food sources on which the flies may develop, and in regions where the species is more common and successful than are the closely related species which are its most immediate competitors. Conversely, the degree of polymorphism is reduced in geographically and ecologically marginal territories, or in territories where the species is less common and less ubiquitous than its relatives and competitors.

Evidence for and against the Hypothesis

Some attempts to test the validity of the above working hypothesis have been made since its original publication. Townsend (1952) reasoned that a species probably occupies relatively few ecological niches near the limits of its distribution area, provided that these limits are not set by impenetrable barriers (such as sea straits or mountain ranges). The amount of polymorphism in marginal areas should, then, be reduced compared to the center of the distribution. He found this to be the case in *D. willistoni* from Florida, where the species reaches its northern limit. On the other hand, White (1951) found no

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diminution of the chromosomal variability towards the distribution margins in the grasshopper *Trimerotropis sparsa*. White has pointed out, however, that in this species the central part of the distribution area may actually be less favorable than some of the marginal ones, being a desert in parts of which the species does not occur at all. Stumm-Zollinger (1953) determined the frequencies of inversion heterozygotes in *Drosophila subobscura* for 8 localities in Switzerland, 4 localities in France, and 1 locality in Portugal. The average numbers of heterozygous inversions per individual female varied in these localities from 2.4 to 4.8, but the author was unable to correlate the variation with any environmental features.

Da Cunha, Brncic, and Salzano (1953) compared the frequencies of inversion heterozygotes in two pairs of closely related species, namely *Drosophila polymorpha* and *D. cardinoides,* and in *D. guaramunu* and *D. griseolineata.* The more widespread, ubiquitous, and common member of each pair proved to be also the more polymorphic one. In *D. nebulosa,* populations which inhabit rich and diversified environments were compared with inhabitants of ecologically and geographically marginal environments. The former populations are more polymorphic than the latter. Mainx, Koske, and Smital (1953) compared the chromosomal variabilities in five European species of the *obscura* group. *D. subobscura,* which is the most widespread and usually also the commonest species, leads with about 40 different gene arrangements recorded. The less common *D. obscura, D. ambigua, D. bifasciata,* and *D. tristis* have respectively 3, 5, 2, and 6 recorded inversions. The number of heterozygous inversions per individual happens however to be high in the only examined strain of the rare *D. tristis.* Even though more extensive studies may change these numbers upward the differences seem to be significant. The authors might have compared their data also with the information available in the literature concerning the American representatives of the *obscura* group. The common *D. pseudoobscura* has (ignoring very rare and endemic chromosomal types) about 14 known inversions, the somewhat less common *D. persimilis* about 8, and the relatively rare *D. miranda* only 2 (Dobzhansky, 1944).

It is obvious that the relationships between the amounts of genetic variability which populations carry and the environments in which they live are complex. Much observation and experimentation is necessary to clarify the situation. The present article reports some new data which bear on the problem at issue. We have attempted to use the hypothesis stated above to predict the approximate amounts of inversion heterozygosis in populations of *D. willistoni* in several selected regions of Brazil which were known to possess certain climatic and biotic characteristics. Collections of population samples of *D. willistoni* were then made in these selected regions, and their chromosomes were examined. In most cases the situation found agreed with the expectation reasonably well; however, the samples from the southern part of the state of Bahia and the adjacent regions gave discordant results. We have also attempted to evaluate the characteristics of the environments in which the collections were made on a quasi-quantitative basis, following some of the methods suggested by Dansereau (1952). Even though these evaluations are admittedly inexact, they make the comparisons of the environments which are inhabited by the populations sampled at least more communicable than such comparisons could be otherwise.

The Critical Regions

Before presenting the detailed data, it may be useful to review briefly the reasons which led to the choice of certain particular geographic regions for investigation. Da Cunha, Burla, and Dobzhansky (1950) found that populations of *D. willistoni* which show the greatest contrast in the amount of chromosomal polymor-

phism live less than 1000 Km apart. Populations of Goyaz and Maranhão may have as many as 9 heterozygous inversions per female on the average, while at Catuni, Bahia, the number is close to 1 (fig. 1 and table 4 in the paper cited above, and fig. 1 and table 3 of the present article, compare localities Nos. 23–26 with No. 21). Catuni is a desert region (caatinga) in which the species is clearly at the limit of its tolerance, while Goyaz and Maranhão have extremely rich floras, combining savannas and gallery forests along the rivers. It seemed important to know whether the fly populations in the intervening territories will show high or low polymorphism in environments approaching the desert type and the savanna-gallery forest type. We have therefore made collections at Barreiras, State of Bahia (locality No. 22 in fig. 1 and table 3), Floriano, State of Piaui (No. 25), and Montes Claros, State of Minas Gerais (No. 17). The first two localities showed a high, and the third a moderate polymorphism in the inhabitants. The expectation was high to moderate polymorphism.

The low polymorphism found in the caatinga desert of Bahia (Catuni, No. 21) was not expected to extend to the coastal zone of Bahia and the forests southward from there (Ilheus, Espirito Santo, Rio Doce, Nos. 19, 18, 16). This prediction proved definitely wrong (fig. 1 and table 3).

A very low average inversion frequency was recorded by da Cunha, Burla, and Dobzhansky (1950) in the population of the peculiar swampy savanna region of northern Marajo Island, where *D. willistoni* is a relatively rare species dominated by its usually much less abundant sibling species, *D. tropicalis*. This fact was confirmed by another sample taken in the same locality in 1952 (locality No. 30). Samples were also taken in the equatorial mainland savannas of Amapa (No. 31) and in forests of Belem and Castanhal (Nos. 28 and 29), where the exceptional ecological situation prevailing on Marajo Island does not obtain. As expected, the polymorphism in these latter samples proved considerably higher than on Marajo (fig. 1 and table 3).

The original samples taken on Rio Negro and Rio Branco (Nos. 33–35) showed large variations in the degree of polymorphism, which were interpreted to reflect the variations in the abundance of *D. willistoni* relative to the competing sibling species (*D. paulistorum, D. equinoxialis,* and *D. tropicalis*). To test the validity of this interpretation, we made collection at other points in equatorial Amazonia, namely at Fordlandia (No. 32) and on the upper Rio Negro (Nos. 36 and 37). As expected, high polymorphism was observed where *D. willistoni* was the dominant species.

Townsend's (1952) finding that the polymorphism is diminished at the northern margin of the distribution area of the species (in Florida) leads to an analogous expectation for the populations of the southern marginal area. Prof. C. Pavan has very kindly collected samples of *D. willistoni* near Buenos Aires in Argentina (Nos. 2 and 3) and Drs. A. Cordeiro and F. N. Salzano in Uruguay and in the southernmost Brazilian State of Rio Grande do Sul (Nos. 4–8). Low polymorphism has been observed in these samples (fig. 1 and table 3), almost exactly matching Townsend's figures. A greater polymorphism was found, as expected, in the subtropical region near Tucuman, Argentina (No. 1). This leads us to expect that *D. willistoni* will be found also in the Andean piedmont south and southeast of Tucuman. The validity of this expectation remains to be tested.

The Inversions

Forty different inversions in the chromosomes of *Drosophila willistoni* have been described and figured by da Cunha, Burla, and Dobzhansky (1950), Dobzhansky, Burla, and da Cunha (1950), and Burla *et al.* (1949). Townsend (1952) has discovered one further inver-

TABLE 1. *Frequencies (in per cent) of inversions in Argentina, Uruguay, and Southern Brazil*

Locality / Inversion	1. Tucuman, Argentina	2. Buenos Aires, Argentina	3. La Plata, Argentina	4. Melo, Uruguay	5. Ponta Grossa, Rio Grande Sul	8. Chapada, Rio Grande Sul
Chromosome XL						
None	80.4	100.0	100.0	96.5	98.1	100.0
D	19.6	—	—	3.5	—	—
F	1.9	—	—	—	—	—
G	1.9	—	—	—	—	—
H	19.6	—	—	3.5	1.9	—
Chromosome XR						
None	88.2	100.0	100.0	100.0	92.4	100 0
A	—	—	—	—	1.8	—
C	9.8	—	—	—	—	—
D	11.7	—	—	—	5.6	—
Females studied	51	24	31	29	53	17
Chromosome II L						
None	23.7	39.3	37.2	26.9	29.8	10.0
A or B	11.0	—	—	4.7	—	—
A + B	1.6	—	—	6.0	—	—
C	6.7	8.9	23.2	25.4	16.3	30.0
D	30.5	8.9	32.5	25.4	29.8	40.0
E	33.0	26.8	11.6	44.8	27.8	46.7
F	36.4	25.0	23.2	22.4	36.5	43.3
H	16.9	14.3	6.9	13.4	16.3	10.0
Chromosome II R						
None	88.2	87.5	100.0	89.5	96.1	90.0
E	11.8	12.5	—	10.5	3.9	10.0
Chromosome III						
None	11.8	17.8	27.9	14.9	19.2	23.3
A	34.7	33.9	23.2	50.7	41.3	50.0
B	55.9	46.4	34.9	34.3	34.6	26.7
F	4.2	1.8	2.3	—	2.8	—
H	9.3	1.8	—	1.5	1.9	6.7
J	36.4	46.4	32.5	55.2	39.4	30.0
L	5.1	—	—	—	—	—
M	0.8	—	—	—	—	—
N	—	—	—	1.5	—	—
Individuals studied	118	56	43	67	104	30

sion in the Florida population. In the considerable new material from South America which we have examined since the above papers were written only a single new inversion has been found—the short inversion N in the third chromosome of a single fly from Uruguay (table 1). This brings the number of inversions known in the species to 42. Although new ones very likely will be discovered when material from regions as yet unexplored is studied, the wide geographic distribution of many chromosomal types in *D. willistoni* (see below) makes it unlikely that the increase will be great.

New data on the geographic distribution of the inversions are summarized in tables 1 and 2. The localities where collections have been made are numbered to correspond with the map in figure 1. The

older data may be found in table 3 of the paper by da Cunha, Burla, and Dobzhansky (1950); the names of the localities correspond to those used in the present article but the numbers do not. A complete list of the collecting localities in South America is given in tables 3 and 4 and figure 1 of the present article. Tables 1 and 2 indicate the percentages of individuals studied which were found heterozygous for a given inversion. Since inversion heterozygotes in the X-chromosomes can be seen only in females, the numbers of individuals studied for X-chromosomes is less than that for the autosomes (tables 1 and 3 in da Cunha, Burla, and Dobzhansky, 1950, are in error giving the numbers of "chromosomes studied"; actually these are the numbers of individuals studied).

Examination of tables 1 and 2 of the present article, together with tables 1 and 3 of that by da Cunha, Burla, and Dobzhansky (1950), shows that the geographic distribution of different inversions may be described under the following three headings:

(1) *Rare and endemic inversions.* Some inversions have been found in a single or in few individuals in single locality. Here belong the inversion IIL–G from central Goyaz, III–K from the same region, Townsend's III–AI from Florida, and III–N from Melo, Uruguay.

(2) *Inversions restricted to a considerable part of the species area.* In the left limb of the X-chromosome, inversion XL–C is so far known only from Central America, XL–E from Costa Rica, and from upper Rio Negro in equatorial Brazil and XL–G from the basin of the Amazon and from Tucuman in Argentina. In the right limb of the X-chromosome, inversion XR–C seems to occur only in parts of the Amazon basin, while XR–E occurs only in southern and central Brazil. In the second chromosome, IIR–D has been recorded from scattered localities on the Rio Negro, in Amapa, in the state of São Paulo, and in Florida. It is possible that more than one cytologically similar but not identical inversion are here involved. In the third chromosome, inversion III–C seems to be confined to the basin of the Amazon, III–D and III–E are rare or absent in southern Brazil, III–G is confined to the interior of the Amazon Valley, III–I to Central Brazil, while III–L and III–M are common in the basin of the Amazon but rare or absent to the south and to the north of it.

(3) *Widely distributed inversions.* Several inversions occur apparently in the whole species distribution area, or are absent in only a small part of it, where they may be discovered by further studies. Thus, IIL–A extends from Florida and Cuba to southern Brazil, but is rare or absent in Rio Grande do Sul, in Uruguay, and near Buenos Aires in Argentina. IIL–E is not only present but also common in almost all populations studied—from Florida to Buenos Aires. The same is true for IIL–F, except that Townsend has encountered it in Cuba but not in Florida. In the third chromosome, the inversion III–A is frequent everywhere from Florida to Buenos Aires, but very remarkably it is absent in the populations of the northern part of the State of Bahia (Nos. 20, 21) and rare in the southern part, in Espirito Santo, and on the Rio Doce (Nos. 16, 18, 19). This is, in fact, a distinctive characteristic of the "Bahia race" (see below). Inversions III–H and III–J extend from Florida to Argentina, the former becoming less frequent to the north and to the south. Inversions III–L and III–M are also widespread but they have not been found in Florida or in Cuba, and they are rare or absent in southernmost Brazil and in Argentina.

The prevalence of geographically widely distributed inversions is an interesting feature of *D. willistoni*. Such wide distributions of inversions have been known only in species associated with man, particularly in *D. melanogaster* and *D. ananassae*. In the latter species the factor responsible is clearly transport of flies by

TABLE 2. *Frequency (in per cent) of inversions in Central and Equatorial Brazil*

Locality / Inversion	14. Boa Esperança, Minas Gerais	15. Rio de Janeiro	16. Rio Doce, Minas Gerais	17. Montes Claros, Minas Gerais	18. Sooretama, Espirito Santo	19. Ilheus, Bahia	20. Salvador, Bahia	21. Catuni, Bahia (1952)	22. Barreiras, Bahia	25. Floriano, Piaui	28. Belem, Para (1952)	29. Castanhal, Para	30. Marajo Island (1952)	31. Ferreira Gomez (Amapa)	32. Fordlandia, Para	33. Rio Negro, Amazonas (1951)	36. Foz de Caiari, Amazonas	37. Içana, Amazonas
Chromosome XL																		
None	33.9	51.2	46.0	49.2	56.1	36.1	32.5	47.6	9.1	51.7	85.1	76.9	100.0	38.5	34.9	33.3	28.6	55.0
A	5.1	7.0	8.0	—	—	3.1	4.6	—	6.8	—	—	—	—	—	—	7.8	—	15.0
B		2.3		—	—	1.0	0.8	—		—	—	—	—	—	3.6	7.8	—	10.0
D	13.5	—	24.0	28.6	21.9	54.6	62.6	52.4	65.9	41.4	9.4	26.9	—	61.4	31.3	62.7	71.5	40.0
E	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3.9	—	—
F	—	2.3	—	1.6	—	1.0	—	—	47.7	31.0	—	11.5	—	28.0	—	19.6	14.3	45.0
G	—	—	—	1.6	—	1.0	—	—	38.6	27.6	—	11.5	—	28.0	—	17.6	14.3	40.0
H	61.0	39.5	30.0	39.7	26.8	—	0.8	—	25.0	22.4	—	3.8	—	—	—	29.4	—	10.0
D + H	—	—	—	—	—	—	—	—	—	—	1.4	—	—	—	2.4	—	—	—
D + F + G	—	—	—	—	—	—	—	—	—	—	4.0	—	—	—	31.3	—	—	—
Chromosome XR																		
None	45.8	27.9	90.0	54.0	100.0	100.0	100.0	100.0	40.9	67.2	90.5	69.2	100.0	84.2	50.6	50.9	57.3	70.0
A	5.1	—	—	6.3	—	—	—	—	18.2	22.4	4.0	7.7	—	15.7	9.6	27.4	14.3	10.0
B		—	—	4.8	—	—	—	—	4.5		—	—	—	—	—	29.4		—
C	1.7	—	—	15.9	—	—	—	—	38.6	25.9	—	—	—	—	—	21.6	28.6	—
D	49.1	58.1	10.0	30.2	—	—	—	—	15.9	3.4	4.0	23.1	—	—	32.5	1.9	14.3	20.0
E	—	41.9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
C + D	—	—	—	—	—	—	—	—	—	—	1.4	—	—	—	8.4	—	—	—
Females studied	59	43	50	63	41	97	123	42	44	58	74	26	36	57	83	51	7	20
Chromosome II L																		
None	19.4	33.0	41.5	35.6	60.6	72.9	88.2	85.9	18.0	24.6	30.4	29.3	67.3	22.5	14.8	24.3	33.3	10.0
A or B	11.2	6.0	3.1	6.9	—	0.5	0.3	—	37.3	29.7	19.6	24.4	12.1	10.8	14.1	42.6	13.3	17.5
A + B	2.0	—	—	1.3	—	—	—	—	20.5	30.5	29.1	14.6	8.6	53.9	40.7	17.5	13.3	25.0
C	1.0	8.0	—	0.6	—	—	—	—	—	—	2.0	7.3	—	—	5.9	—	—	10.0
D	22.5	41.0	43.1	31.2	33.7	1.4	4.6	1.6	20.5	2.5	—	2.4	—	—	3.7	28.1	20.0	10.0
E						24.8	7.8	10.9			8.8	12.2	—	5.9	22.2	2.9		25.0
D + E	17.4	8.0	10.0	19.4	4.4	—	—	—	2.4	0.8	—	—	—	—	—	—	13.3	—
F	64.3	33.0	8.5	16.9	2.2	0.5	3.6	3.1	30.1	35.6	35.1	46.3	19.0	31.4	40.0	29.1	26.7	47.5
H	10.2	1.0	—	0.6	—	—	—	—	3.6	4.2	—	—	—	—	1.5	10.7	33.3	15.0

H	10.2	1.0	—	0.6	—	—	—	—	3.6	4.2	—	—	—	—	1.5	10.7	33.3	15.0

TABLE 2.—(*Continued*)

Locality / Inversion	14. Boa Esperança, Minas Gerais	15. Rio de Janeiro	16. Rio Doce, Minas Gerais	17. Montes Claros, Minas Gerais	18. Sooretama, Espírito Santo	19. Ilheus, Bahia	20. Salvador, Bahia	21. Catuni, Bahia (1952)	22. Barreiras, Bahia	25. Floriano, Piauí	28. Belem, Para (1952)	29. Castanhal, Para	30. Marajo Island (1952)	31. Ferreira Gomez (Amapa)	32. Fordlandia, Para	33. Rio Negro, Amazonas (1951)	36. Foz de Caiari, Amazonas	37. Içana, Amazonas
Chromosome II R																		
None	79.6	97.0	98.5	83.1	100.0	96.8	96.8	96.9	60.2	93.2	89.2	78.0	96.5	85.2	78.5	88.3	74.3	47.5
A	—	—	—	—	—	—	0.3	—	—	—	1.4	—	—	—	5.2	7.8	—	17.5
B	—	—	—	—	—	—	—	—	1.4	—	—	2.4	—	—	0.4	7.8	—	2.5
C	—	—	—	—	—	1.8	—	—	—	—	0.7	4.9	—	2.9	4.4	—	—	12.5
D	—	—	—	—	—	1.4	—	—	—	—	1.0	—	—	1.0	—	—	—	22.5
E	20.4	3.0	1.5	16.9	—		2.8	3.1	38.5	6.8	8.8	14.6	3.5	10.7	11.9	9.7	33.3	10.0
F	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2.9	6.7	—
Chromosome III																		
None	24.5	67.0	88.5	61.9	94.4	92.6	85.3	78.1	3.6	24.6	33.8	16.1	41.3	14.7	10.9	17.5	13.3	27.5
A or B	51.0	19.0	2.3	18.1	1.1	1.8	—	—	39.8	29.7	23.6	16.1	1.7	23.5	22.2	35.8	60.0	35.0
A + B	4.1	—	—	5.6	—	1.4	—	—	4.8	8.5	5.4	6.5	—	2.0	10.9	7.6	—	12.5
C	—	—	—	—	—	—	—	—	2.4	—	18.2	4.8	—	—	—	—	—	5.0
D	3.1	1.0	—	3.8	1.1	—	—	—	32.5	37.3	7.4	38.7	34.5	41.2	27.5	1.9	—	20.0
E	—	—	—	—	—	—	—	—	—	—	2.0	3.2	—	2.0	1.5	—	—	5.0
F	7.1	—	—	8.8	—	0.5	1.1	—	38.5	21.2	30.4	41.9	32.8	42.2	21.5	29.1	53.3	30.0
G	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.7	—	—	—
H	16.3	2.0	—	21.3	—	0.5	0.3	3.1	39.0	26.3	7.4	4.8	6.9	8.8	54.7	55.3	53.3	50.0
I	—	—	—	0.6	—	—	—	—	2.4	—	—	—	—	—	0.7	—	—	—
J	39.8	22.0	10.8	11.2	3.3	4.1	13.9	21.9	8.4	36.4	37.8	27.4	39.7	44.1	19.2	13.4	33.3	20.0
L	13.3	—	—	20.0	—	—	—	—	62.6	14.4	2.0	1.6	—	3.9	19.2	28.1	20.0	20.0
M	1.0	—	—	6.2	—	—	—	—	34.9	3.4	—	—	—	—	5.9	—	—	12.5
Individuals studied	100	98	130	160	89	218	280	64	83	118	148	62	58	102	135	103	15	40

TABLE 2. *Frequency (in per cent) of inversions in Central and Equatorial Brazil*

Locality / Inversion	14. Boa Esperança, Minas Gerais	15. Rio de Janeiro	16. Rio Doce, Minas Gerais	17. Montes Claros, Minas Gerais	18. Sooretama, Espirito Santo	19. Ilheus, Bahia	20. Salvador, Bahia	21. Catuni, Bahia (1952)	22. Barreiras, Bahia	25. Floriano, Piaui	28. Belem, Para (1952)	29. Castanhal, Para	30. Marajo Island (1952)	31. Ferreira Gomez (Amapa)	32. Fordlandia, Para	33. Rio Negro, Amazonas (1951)	36. Foz de Caiari, Amazonas	37. Içana, Amazonas
Chromosome XL																		
None	33.9	51.2	46.0	49.2	56.1	36.1	32.5	47.6	9.1	51.7	85.1	76.9	100.0	38.5	34.9	33.3	28.6	55.0
A	5.1	7.0	8.0	—	—	3.1	4.6	—	6.8	—	—	—	—	—	—	7.8	—	15.0
B		2.3		—	—	1.0	0.8	—		—	—	—	—	—	3.6	7.8	—	10.0
D	13.5	—	24.0	28.6	21.9	54.6	62.6	52.4	65.9	41.4	9.4	26.9	—	61.4	31.3	62.7	71.5	40.0
E	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3.9	—	—
F	—	2.3	—	1.6	—	1.0	—	—	47.7	31.0	—	11.5	—	28.0	—	19.6	14.3	45.0
G	—	—	—	1.6	—	1.0	—	—	38.6	27.6	—	11.5	—	28.0	—	17.6	14.3	40.0
H	61.0	39.5	30.0	39.7	26.8	—	0.8	—	25.0	22.4	—	3.8	—	—	—	29.4	—	10.0
D + H	—	—	—	—	—	—	—	—	—	—	1.4	—	—	—	2.4	—	—	—
D + F + G	—	—	—	—	—	—	—	—	—	—	4.0	—	—	—	31.3	—	—	—
Chromosome XR																		
None	45.8	27.9	90.0	54.0	100.0	100.0	100.0	100.0	40.9	67.2	90.5	69.2	100.0	84.2	50.6	50.9	57.3	70.0
A	5.1	—	—	6.3	—	—	—	—	18.2	22.4	4.0	7.7	—	15.7	9.6	27.4	14.3	10.0
B		—	—	4.8	—	—	—	—	4.5		—	—	—	—	—	29.4		—
C	1.7	—	—	15.9	—	—	—	—	38.6	25.9	—	—	—	—	—	21.6	28.6	—
D	49.1	58.1	10.0	30.2	—	—	—	—	15.9	3.4	4.0	23.1	—	—	32.5	1.9	14.3	20.0
E	—	41.9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
C + D	—	—	—	—	—	—	—	—	—	—	1.4	—	—	—	8.4	—	—	—
Females studied	59	43	50	63	41	97	123	42	44	58	74	26	36	57	83	51	7	20
Chromosome II L																		
None	19.4	33.0	41.5	35.6	60.6	72.9	88.2	85.9	18.0	24.6	30.4	29.3	67.3	22.5	14.8	24.3	33.3	10.0
A or B	11.2	6.0	3.1	6.9	—	0.5	0.3	—	37.3	29.7	19.6	24.4	12.1	10.8	14.1	42.6	13.3	17.5
A + B	2.0	—	—	1.3	—	—	—	—	20.5	30.5	29.1	14.6	8.6	53.9	40.7	17.5	13.3	25.0
C	1.0	8.0	—	0.6	—	—	—	—	—	—	2.0	7.3	—	—	5.9	—	—	10.0
D	22.5	41.0	43.1	31.2	33.7	1.4	4.6	1.6	20.5	2.5	—	2.4	—	—	3.7	28.1	20.0	10.0
E						24.8	7.8	10.9			8.8	12.2	—	5.9	22.2	2.9		25.0
D + E	17.4	8.0	10.0	19.4	4.4	—	—	—	2.4	0.8	—	—	—	—	—	—	13.3	—
F	64.3	33.0	8.5	16.9	2.2	0.5	3.6	3.1	30.1	35.6	35.1	46.3	19.0	31.4	40.0	29.1	26.7	47.5
H	10.2	1.0	—	0.6	—	—	—	—	3.6	4.2	—	—	—	—	1.5	10.7	33.3	15.0

H	10.2	1.0	—	0.6	—	—	—	—	3.6	4.2	—	—	—	—	1.5	10.7	33.3	15.0

TABLE 2.—(*Continued*)

Locality / Inversion	14. Boa Esperança, Minas Gerais	15. Rio de Janeiro	16. Rio Doce, Minas Gerais	17. Montes Claros, Minas Gerais	18. Sooretama, Espirito Santo	19. Ilheus, Bahia	20. Salvador, Bahia	21. Catuni, Bahia (1952)	22. Barreiras, Bahia	25. Floriano, Piaui	28. Belem, Para (1952)	29. Castanhal, Para	30. Marajo Island (1952)	31. Ferreira Gomez (Amapa)	32. Fordlandia, Para	33. Rio Negro, Amazonas (1951)	36. Foz de Caiari, Amazonas	37. Içana, Amazonas
Chromosome II R																		
None	79.6	97.0	98.5	83.1	100.0	96.8	96.8	96.9	60.2	93.2	89.2	78.0	96.5	85.2	78.5	88.3	74.3	47.5
A	—	—	—	—	—	—	0.3	—	—	—	1.4	—	—	—	5.2	7.8	—	17.5
B	—	—	—	—	—	—	—	—	1.4	—	—	2.4	—	—	0.4	7.8	—	2.5
C	—	—	—	—	—	1.8	—	—	—	—	0.7	4.9	—	2.9	4.4	—	—	12.5
D	—	—	—	—	—	1.4	—	—	—	—	1.0	—	—	1.0	—	—	—	22.5
E	20.4	3.0	1.5	16.9	—		2.8	3.1	38.5	6.8	8.8	14.6	3.5	10.7	11.9	9.7	33.3	10.0
F	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2.9	6.7	—
Chromosome III																		
None	24.5	67.0	88.5	61.9	94.4	92.6	85.3	78.1	3.6	24.6	33.8	16.1	41.3	14.7	10.9	17.5	13.3	27.5
A or B	51.0	19.0	2.3	18.1	1.1	1.8	—	—	39.8	29.7	23.6	16.1	1.7	23.5	22.2	35.8	60.0	35.0
A + B	4.1	—	—	5.6	—	1.4	—	—	4.8	8.5	5.4	6.5	—	2.0	10.9	7.6	—	12.5
C	—	—	—	—	—	—	—	—	2.4	—	18.2	4.8	—	—	—	—	—	5.0
D	3.1	1.0	—	3.8	1.1	—	—	—	32.5	37.3	7.4	38.7	34.5	41.2	27.5	1.9	—	20.0
E	—	—	—	—	—	—	—	—	—	—	2.0	3.2	—	2.0	1.5	—	—	5.0
F	7.1	—	—	8.8	—	0.5	1.1	—	38.5	21.2	30.4	41.9	32.8	42.2	21.5	29.1	53.3	30.0
G	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.7	—	—	—
H	16.3	2.0	—	21.3	—	0.5	0.3	3.1	39.0	26.3	7.4	4.8	6.9	8.8	54.7	55.3	53.3	50.0
I	—	—	—	0.6	—	—	—	—	2.4	—	—	—	—	—	0.7	—	—	—
J	39.8	22.0	10.8	11.2	3.3	4.1	13.9	21.9	8.4	36.4	37.8	27.4	39.7	44.1	19.2	13.4	33.3	20.0
L	13.3	—	—	20.0	—	—	—	—	62.6	14.4	2.0	1.6	—	3.9	19.2	28.1	20.0	20.0
M	1.0	—	—	6.2	—	—	—	—	34.9	3.4	—	—	—	—	5.9	—	—	12.5
Individuals studied	100	98	130	160	89	218	280	64	83	118	148	62	58	102	135	103	15	40

TABLE 3. *Mean numbers of heterozygous inversions per individual in different localities*

Locality	Mean	Limits	Locality	Mean	Limits
1. Tucuman, Argentina	♀3.82±0.31	1–8	23. Monjolinho, Goyaz	♀9.36±0.26	2–14
	♂2.76±0.19	0–7		♂6.56±0.31	2–10
2. Buenos Aires, Argentina	♀♂2.28±0.19	0–5	24. Palma, Goyaz	♀9.11±0.44	3–16
				♂5.57±0.36	2–9
3. Delta de la Plata, Argentina	♀♂1.93±0.17	0–5	25. Floriano, Piaui	♀5.14±0.33	0–11
				♂3.18±0.22	0–8
4. Melo, Uruguay	♀2.83±0.33	0–5	26. Carolina, Maranhão	♀8.65±0.58	4–13
	♂3.03±0.20	0–6		♂4.33±0.42	1–7
5. Ponta Grossa, Rio Grande Sul	♀2.82±0.19	0–7	27. Imperatriz, Maranhão	♀5.47±0.37	1–12
	♂2.33±0.16	0–5		♂3.24±0.21	1–6
6. Reuter, Rio Grande Sul	♀2.90±0.75	0–7	28. Belem, Para (1949)	♀2.85±0.18	0–7
	♂2.40±0.53	1–4		♂2.53±0.23	0–7
7. Santo Angelo, Rio Grande Sul	♀1.90±0.24	0–4	28. Belem, Para (1952)	♀3.16±0.16	0–7
	♂1.25±0.42	0–3		♂2.75±0.17	0–6
8. Chapada and Feliz, Rio Grande Sul	♀♂3.02±0.37	0–6	29. Castanhal, Para	♀4.42±0.34	0–8
				♂3.00±0.20	0–6
9. Iguassu, Parana	♀3.20±0.19	0–8	30. Marajo Island (1949)	♀2.05±0.48	0–7
	♂2.81±0.22	0–7		♂0.93±0.43	0–5
10. Paranagua, Parana	♀2.61±0.39	0–5	30. Marajo Island (1952)	♀♂1.67±0.19	0–6
	♂2.50±0.68	0–4			
11. Vila Atlantica, São Paulo	♀3.06±0.12	0–9	31. Ferreira Gomez, Amapa	♀4.75±0.21	1–7
	♂2.34±0.12	0–7		♂3.42±0.21	1–6
12. Mogi das Cruzes, São Paulo	♀3.97±0.13	0–13	32. Fordlandia, Para	♀5.88±0.20	3–11
	♂2.92±0.34	0–9		♂4.00±0.16	2–6
13. Pirassununga, São Paulo	♀4.13±0.10	0–11	33. Rio Negro, Amazonas (1949)	♀5.03±0.49	1–9
	♂3.52±0.13	0–8		♂4.03±0.36	0–7
14. Boa Esperança, Minas Gerais	♀4.34±0.25	0–9	33. Rio Negro, Amazonas (1952)	♀5.72±0.34	0–12
	♂3.20±0.28	1–7		♂3.31±0.24	0–7
15. Rio de Janeiro, D.F.	♀2.91±0.22	0–4	34. Savanna, Rio Branco	♀4.77±0.43	0–11
	♂1.63±0.15	0–3		♂3.00±0.23	0–7
16. Upper Rio Doce, Minas Gerais	♀1.50±0.15	0–5	35. Mucajai, Rio Branco	♀2.67±0.46	0–8
	♂0.96±0.08	0–3		♂2.50±0.49	0–5
17. Montes Claros, Minas Gerais	♀3.54±0.28	0–10	36. Foz de Caiari, Amazonas	♀5.29±0.51	2–7
	♂2.07±0.17	0–8		♂4.25±0.78	1–7
18. Sooretama, Espirito Santo	♀1.03±0.17	0–4	37. Içana, Amazonas	♀6.74±0.56	4–11
	♂0.52±0.09	0–2		♂4.65±0.28	2–6
19. Ilheus, Bahia	♀1.03±0.07	0–3	38. Japiim-Moa, Acre	♀4.68±0.26	0–9
	♂0.40±0.05	0–2		♂4.58±0.41	0–8
20. Salvador, Bahia	♀1.06±0.09	0–5	39. Palmares, Acre	♀5.49±0.31	1–9
	♂0.35±0.10	0–3		♂4.86±0.35	1–9
21. Catuni, Bahia (1949)	♀0.81±0.03	0–5	40. Porto Velho, Guapore	♀6.39±0.66	1–10
	♂0.42±0.05	0–2		♂5.25±0.99	3–7
21. Catuni, Bahia (1952)	♀1.05±0.12	0–3	41. Santa Cruz, Bolivia	♀5.50±0.73	3–8
	♂0.32±0.10	0–1		♂3.00±1.22	0–7
22. Barreiras, Bahia	♀7.36±0.40	2–12			
	♂4.85±0.25	2–9			

man and introduction of populations from one country to another. In wild species the populations of different parts of the species area are usually racially distinct, and differences in relative frequencies of chromosomal types are among the race characteristics. Thus, no gene arrangement in the third chromosome occurs in all parts of the distribution area of *D. pseudoobscura,* although the area occupied by this species is less extensive than that of *D. willistoni.* Since, at least in Brazil, *D. willistoni* is not closely associated with man, there is no reason to think that transport by man has played any role in the spread of its chromosomal variants. No

matter where in the distribution area of *D. willistoni,* a given inversion, such as IIL–E or III–J, may have first arisen, the inversion must have traveled over enormous distances and must have overcome formidable distribution barriers to achieve a spread from Florida to Argentina. As pointed out by Townsend (1952) this shows that a genetic trait favored by natural selection may be able to spread and penetrate populations of a species living in remote parts of the species area. Unless one supposes that the whole species with all its widespread inversions arose in a limited area and then invaded extensive new territories, it follows that an "evolutionary invention" appearing anywhere in the distribution region of a species may become a property of the species as a whole.

Frequency of Inversion Heterozygotes

The mean numbers of heterozygous inversions per individual are summarized in table 3. The old data (da Cunha, Burla, and Dobzhansky, 1950) as well as the new

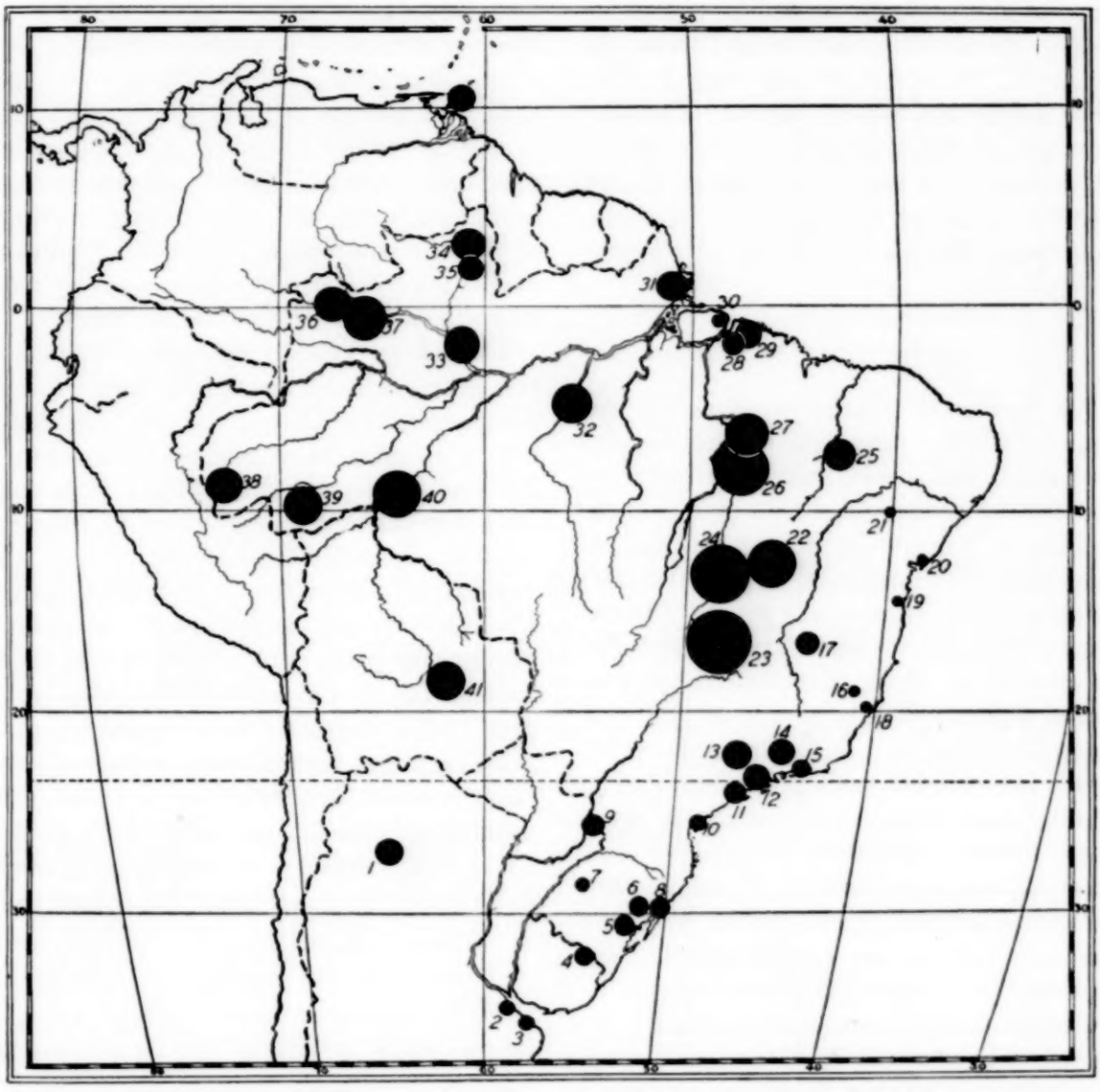

Fig. 1. The collecting localities in South America at which samples of populations of *Drosophila willistoni* have been collected. The localities are numbered as in tables 1–4 and in figures 1–3. The diameters of the black circles are proportional to the mean numbers of heterozygous inversions per female fly.

are here included. For most localities separate figures are given for females and for males; this is, of course, because the inversions in the X-chromosomes can not be detected in the males. A single figure for both sexes is given only for populations in which inversion heterozygotes involving the X-chromosome are absent. The mean frequencies of heterozygous inversions per female are also represented in the form of a map in figure 1; the diameters (not the areas!) of the circles are proportional to the frequencies observed.

The degree of inversion heterozygosis varies, in females, from an average of about 1 in the State of Bahia (localities 19–21) to about 9 in Goyaz (Nos. 23 and 24). In males, the average is 0.3–0.4 in Bahia and 5–6 in Goyaz. The distribution of the populations with different amounts of polymorphism in South America is shown in figure 1. For data concerning Florida and Cuba see Townsend (1952).

Evaluation of the Environment

We may now inquire whether the genetic variability observed in the populations of *D. willistoni* is correlated with the diversity of ecological niches which these populations occupy in the geographic regions which they inhabit. The difficulties facing such an inquiry are great. On the genetic side, chromosomal polymorphism, the amount of which is estimated by cytological studies, is only a part of the total genetic variability. A population which shows no chromosomal polymorphism at all may nevertheless be genetically polymorphic and ecologically polyvalent. On the ecological side, there is no satisfactory method at present to determine with any precision the number of ecological niches exploited by a species or a population.

Certain common sense propositions can of course be stated. A species of Drosophila which feeds on fruits encounters a greater variety of ecological opportunities in a territory with a diversified flora of fruit trees than in a country which yields only few kinds of fruits. The relations between Drosophila and their food sources are, however, so complex (da Cunha, Dobzhansky, and Sokoloff, 1951; Dobzhansky and da Cunha, 1954) that the number of species of fruits available is not a reliable measure of the diversity of even the nutritional aspects of the environment which the flies meet. A country with a seasonally variable climate demands a greater ecological versatility from its inhabitants than a country with an equable climate. The response to these demands may however involve either development of a genetic polyvalence in the population or a restriction of the biotic activity to some seasons and quiescence at other seasons. Marginal parts of the distribution area of a species are likely to have fewer ecological niches occupied by the species than the central parts. However the biotic characteristics of different portions of the distribution area are also relevant at this point. The distribution center may actually be unfavorable to the species.

Dansereau (1952) has justly criticized the geneticists, because they "refer to environment, but neither describe or measure it, or relate it to other environments." He has then proceeded to act in a constructive way unusual for a critic, namely to develop methods of description of environments in quasi-quantitative terms. His methods consist essentially in making evaluations of as many physical and biotic aspects of the environment as seem relevant to the organism under consideration. Although Dansereau's own work concerns plant species, his ideas are applicable to other organisms, and they have guided us in the following attempts to evaluate the environments which *D. willistoni* faces in different parts of its distribution area.

To evaluate the bearing of climatic factors on the ecological adjustment of populations of *D. willistoni,* we have used the classification of the climates of Brazil and of South America proposed by James (1942) and by Serebrenick (1945). Following the lead of other meteorologists,

these authors distinguish in the territory inhabited by our species tropical climates, symbolized by T, and temperate ones, denoted by t in table 4. Since *D. willistoni* is a species most at home in the tropical zone, we assign to T climates the value of 1, and to t climates the value of 0. Seasonally uniform climates (iso-climates) are symbolized by i, and seasonally changeable ones (aniso-climates) by omission of that letter. On the assumption that adaptive polymorphism is one of the ways the organism can use to adjust itself to periodic alterations in its environment, aniso-climates are assigned the value of 1 and iso-climates of 0. Furthermore, superhumid ($\bar{U}$), humid (U), semiarid (u), and arid (a) climates are distinguished, and the values of 1, 2, 1, and 0 respectively are assigned to them in table 4.

A classification of the biotic provinces

TABLE 4. *Evaluations of the environment in the localities where collections of* Drosophila willistoni *have been made*

Locality	Climate		Biotic	Competitors	Fidelity	Total Rating
1. Tucuman, Argentina	tU	-3	2	4	0	9
2. Buenos Aires, Argentina	tiU	-2	1	4	0	7
3. Delta de la Plata, Argentina	tiU	-2	1	4	0	7
4. Melo, Uruguay	tiU	-2	1	4	0	7
5. Ponta Grossa, Rio Grande Sul	tiu	-1	1½	4	0	6½
6. Reuter, Rio Grande Sul	tiu	-1	1½	4	0	6½
7. Santo Angelo, Rio Grande Sul	tu	-2	1½	4	0	7½
8. Chapada, Rio Grande Sul	tiu	-1	1½	4	0	6½
9. Iguassu, Parana	tiu	-1	2	4	0	7
10. Paranagua, Parana	Ti$\bar{U}$	-2	2	3	0	7
11. Vila Atlantica, São Paulo	Ti$\bar{U}$	-2	2	2	1	7
12. Mogi, São Paulo	tU	-3	2	2	0	7
13. Pirassununga, São Paulo	tu	-3	2	4	0	9
14. Boa Esperanza, Minas Gerais	tu	-3	2	4	0	9
15. Rio de Janeiro	Ti$\bar{U}$	-2	2	2	1	7
16. Rio Doce, Minas Gerais	TiU	-3	2	4	0	9
17. Montes Claros, Minas Gerais	Tu	-3	2	4	0	9
18. Sooretama, Espirito Santo	TiU	-3	2	3	1	9
19. Ilheus, Bahia	TiU	-3	2	4	0	9
20. Salvador, Bahia	Tiu	-2	1	4	0	7
21. Catuni, Bahia	Ta	-1	1	4	0	6
22. Barreiras, Bahia	Tu	-3	3	3	0	9
23. Monjolinho, Goyaz	TU	-4	4	3	0	11
24. Palma, Goyaz	TU	-4	4	4	0	12
25. Floriano, Piaui	TU-Tu	-3½	2	3	0	8½
26. Carolina, Maranhao	TU	-4	4	3	0	11
27. Imperatriz, Maranhao	TU	-4	4	4	0	12
28. Belem, Para	T$\bar{U}$	-3	3	2	1	9
29. Castanhal, Para	T$\bar{U}$	-3	3	2	1	9
30. Marajo Island	T$\bar{U}$	-3	1	1½	0	5½
31. Ferreira Gomez, Amapa	T$\bar{U}$	-3	4	3	1	11
32. Fordlandia, Para	TU	-4	3	2	1	10
33. Rio Negro, Amazonas	Ti$\bar{U}$	-2	3	2	1	8
34. Savanna, Rio Branco	TU	-4	3½	3	0	10½
35. Mucajai, Rio Branco	T$\bar{U}$	-3	3	1	1	8
36. Foz de Caiari, Amazonas	Ti$\bar{U}$	-2	4	1	1	8
37. Içana, Amazonas	Ti$\bar{U}$	-2	4	2	1	9
38. Japiim-Moa, Acre	T$\bar{U}$	-3	3	2	1	9
39. Palmares, Acre	T$\bar{U}$	-3	3	2	1	9
40. Porto Velho, Guapore	T$\bar{U}$	-3	3	2	1	9
41. Santa Cruz, Bolivia	tu	-2	3	3	1	9

of South America is borrowed from James (1942) and Sampaio (1945), supplemented by our own observations on the regions in which we have traveled and collected the Drosophila material. As far as *D. willistoni* is concerned, we distinguish (a) rigorous environments, to which the value of 1 is assigned, (b) moderate environments having the value 2, (c) rich environments with the value of 3, and (d) luxuriant environments, 4. Under (a) we include the deserts of caatinga, open grasslands (steppe, pampa), and the extensive swamps of the northern Marajo Island. The coastal forests of Brazil, extending from southern Bahia to Rio Grande do Sul, are included under (b), while most of the Amazonian hylaea is under (c). The region of the upper Rio Negro, where several facies of the hylaea rainforest extremely rich in plant species occur side by side, and the southern part of the Amazon Basin, where the rich savanna and gallery forest associations alternate mosaic-fashion, are put under (d).

The biotic factor which we believe to be of major consequence in the ecology of *D. willistoni* is the presence of competing Drosophila species in the same geographic region. Just which species are most important as competitors is not known. We have assumed that the very closely related (sibling) species *D. paulistorum, D. equinoxialis,* and *D. tropicalis* are most likely to be involved. Accordingly, a value of 4 is assigned to regions in which *D. willistoni* is predominant (75–100% of the total catch of the four sibling species), a value 3 where *D. willistoni* amounts to 50–75%, a value of 2 where it amounts to 25–50%, and a value of 1 where only 0–25% of *D. willistoni* is found.

Finally, we followed Dansereau (1952) and other plant ecologists in considering the "fidelity" of *D. willistoni.* This notion connotes restriction of the organism to a certain aspect of the environment (stenokous or stenoecic forms), or its ability to permeate the entire environment (euryokous, euryoecic). A value of 1 is assigned to low, and of 0 to high, fidelity. The estimate of the fidelity is based on

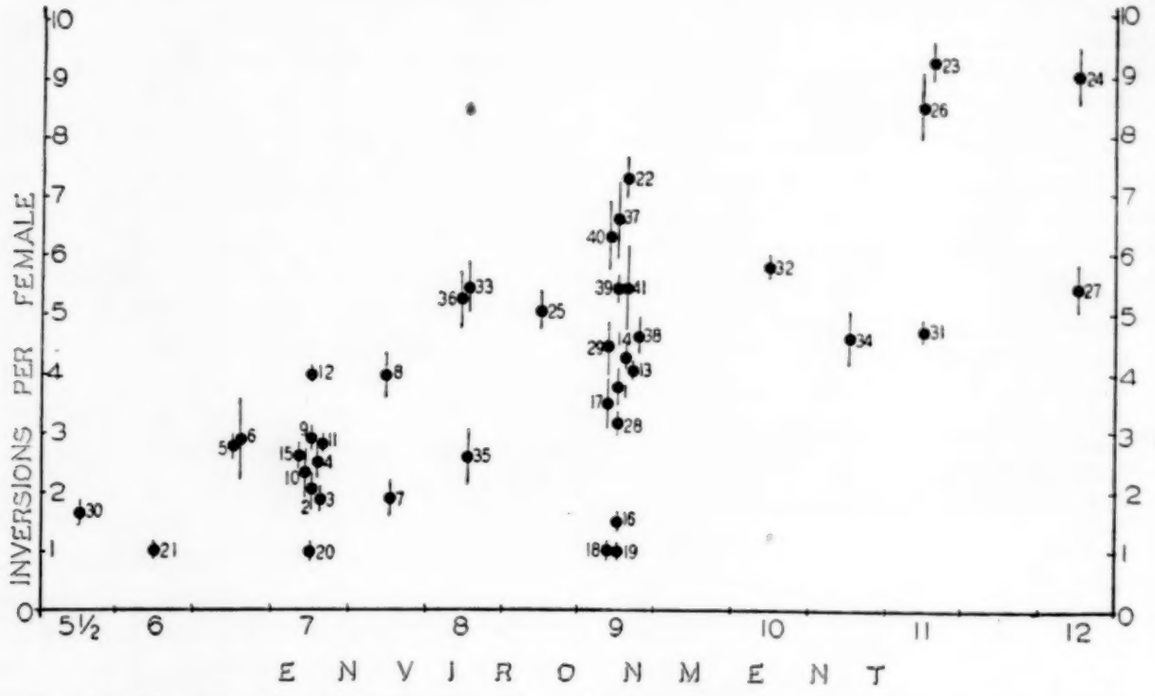

FIG. 2. Mean numbers of heterozygous inversions per female correlated with estimates of the heterogeneity of the environments. The vertical lines indicate the limits of two standard errors of the mean numbers of heterozygous inversions. The localities are numbered to correspond to tables 1–4 and to figures 1 and 3.

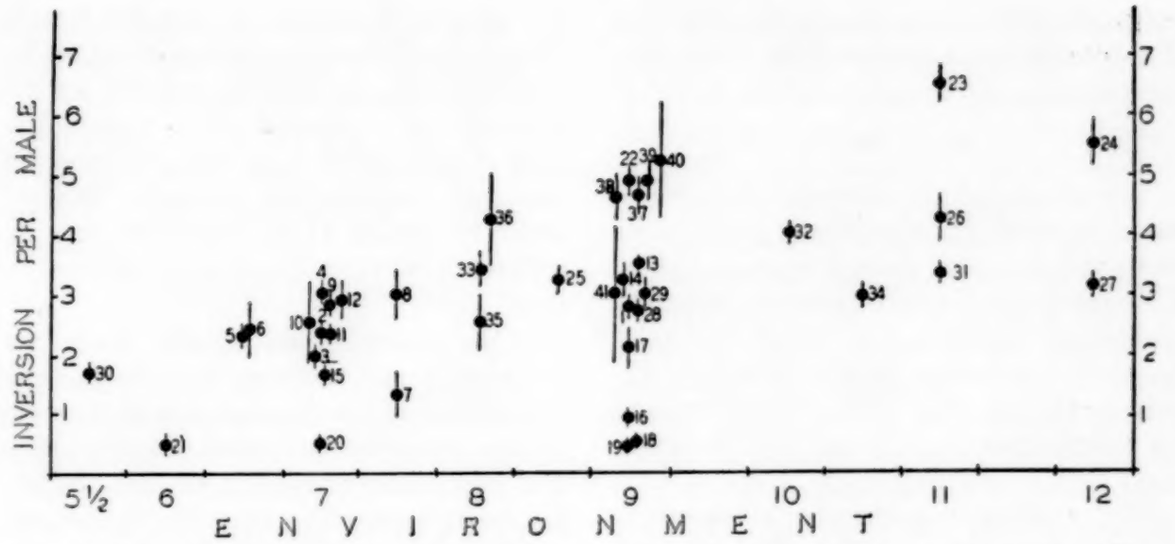

FIG. 3. Mean numbers of heterozygous inversion per male correlated with estimates of the heterogeneity of the environments.

our collecting experience, which showed the species to be more or less omnipresent or confined to some habitats in a given region, especially showing large seasonal changes in population size or a more or less evenly abundant population.

The evaluations are summarized in table 4, which also shows the total or combined ratings of the environments in the regions where collecting was made.

POLYMORPHISM AND ENVIRONMENT CORRELATED

The environment ratings (abscissae) and the average numbers of heterozygous inversions per individual (ordinates) are shown in figures 2 and 3 in the form of scatter diagrams, separately for females and for males. A positive correlation is apparent, despite the wide and statistically significant variations of the average numbers of inversions in localities with the same environmental ratings. Such variations are certainly not unexpected, considering the admittedly rough and incomplete evaluations of the environmental variables.

Three localities, Nos. 16, 18, and 19, make the correlations much less perfect than they would be otherwise (figs. 2 and 3). In these localities the polymorphism is very low, and yet their environmental ratings are fairly high. A glance on the map (fig. 1) shows that the three localities (upper Rio Doce, Sooretama, and Ilheus) are geographically close to each other. They lie in the coastal tropical forests of southern Bahia and in the adjacent parts of the States of Espirito Santo and Minas Gerais. Despite the rich flora of these forests, and despite *D. willistoni* being here the dominant species far outnumbering its siblings, the chromosomal polymorphism is as limited as it is in the extremely rigorous environment of the caatinga desert (No. 21) and the relatively rigorous one of the drier coast of Bahia at Salvador (No. 20). All these localities (Nos. 16, 18–21) are inhabited by a chromosomally fairly uniform population, which may be provisionally referred to as the Bahia race. It is an open question how far this race extends northwards in the Brazilian Northeast, where no collections have been made. As pointed out above, another characteristic of this race seems to be the scarcity of the inversion III–A, which occurs throughout the species. The Bahia race also lacks, or shows low frequencies of, inversions in the right limb of the X-chromosome, in the right limb of the second chromosome, and some inversions in the third chromosome, which happen to be rare or absent also in the

geographically quite remote populations of southernmost Brazil and the Argentine pampa.

Conclusions

The relationships between the genetic polymorphism in Mendelian populations and environmental diversity are complex. This could hardly be otherwise, since the number of variables involved in this relationship is certainly great. We are still only exploring this field of studies, and the conclusions reached can only be tentative.

The working hypothesis that polymorphism is basically a method which a population can use to become adapted to a multiform environment is supported by the evidence so far available. Most or all chromosomal inversions found in actual populations of Drosophila are maintained by an adaptive superiority of the heterozygotes. This is balanced polymorphism. It should, however, be noted that natural selection will maintain in a panmictic population a multiplicity of genotypes, provided that each of the latter has a net advantage over all others in at least one ecological niche in the territory inhabited by the population. This important postulate has been demonstrated mathematically by Levene (1953). He shows that, at least granting certain simplifying assumptions, the maintenance of a variety of genotypes in a panmictic population obeys the same rules which apply to non-interbreeding populations (species) or to clones.

Ludwig (1950) has reached a conclusion similar to that of Levene for a less general case, namely for an "ecomutation" which can use a food source which the parental form can not use.[2] A mutant of this sort will be maintained in the population by natural selection even if it is far less efficient than the parental form in the ecological niches exploited by the latter. A heterogeneous environment furnishes a stimulus which acts towards increasing the genetic diversity of the inhabitants. Thoday (1953) has expressed somewhat similar views, in that he regards genetic diversity as a method of increasing the hold of a population on the environment, and thus contributing towards "fitness," which he defines as the ability to leave descendants after a prolonged interval of time.

It is, nevertheless, hardly to be expected that our working hypothesis could alone account for the immense complexity of the interrelations between variability and environment found in nature. Among the facts brought to light in the present article, it has failed to explain the chromosomal uniformity of the Bahia race, which inhabits not only the severe environment of the caatinga desert but also the exuberant coastal forests to the south of the desert. A possible explanation of this situation may be sought along the lines suggested by Voipio (1952). Regardless of whether the area inhabited by the species as a whole is expanding or contracting, local populations which have achieved an adaptive advantage may overflow into neighboring territories and supplant indigenous populations. It is, then, possible that an "evolutionary invention" has appeared among the relatively uniform inhabitants of the caatinga and that the resulting race is in the process of overrunning the more luxuriant environments of the coastal forests. Such an "evolutionary invention" may start a new cycle of genetic diversification, provided that the genetic basis of this "invention" is connected with certain linked gene complexes, the high adaptive value of which makes the population temporarily genetically uniform. A similar situation may have arisen in the race of *Drosophila pseudoobscura* in the Great Basin of the Western United States; the chromosomal uniformity of this race contrasts with the heterogeneity of the populations living in surrounding territories on all sides.

Finally, it should be remembered that genetic diversification within Mendelian populations is only one of the methods

[2] Ludwig ascribes the discovery of such "ecomutations" to Turesson and, of all people, to Lysenko.

whereby a diversified environment can be mastered. The alternative is cleavage of the population into two or more reproductively more or less isolated ones, in other words differentiation of species. Both methods have been used extensively in evolution.

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Summary

The purpose of the present study has been to test further the working hypothesis that the genetic polymorphism in Mendelian populations enables them to exploit a greater variety of adaptive niches than could be within reach of genetically more uniform populations. The chromosomal variability has been used as an index of the degree of genetic polymorphism in populations of *Drosophila willistoni*.

Samples of natural populations of *D. willistoni* have been collected in four regions in South America, for which expectations of the degrees of polymorphism to be found in the populations of the species in question were formulated on the basis of the above working hypothesis and of data secured earlier (by da Cunha, Burla, and Dobzhansky, 1950). The expectations were confirmed in three regions, and contradicted in the fourth.

The diversity of adaptive niches occupied by *D. willistoni* in different regions has been evaluated in quasi-quantitative terms, following methods suggested by Dansereau (1952). The values obtained show a positive correlation with the degrees of polymorphism in the populations of the fly (figs. 2 and 3). This correlation would have been more pronounced than it is if it were not for the populations which inhabit the tropical coastal forests in the State of Bahia and adajacent territories. These exceptional populations are chromosomally more uniform than expected in the rich and diversified environments in which they live.

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COMPARABLE MUTANT EYE COLORS IN MORMONIELLA AND PACHYCREPOIDEUS (*HYMENOPTERA: PTEROMALIDAE*)

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INTRODUCTION

In such forms as Drosophila and Habrobracon visible mutations from the wild type are, for the most part, recessive and each change is usually thought to be of a simple nature involving modification of a single trait. Further study, however, reveals that minor modifications often accompany the major difference, that less obvious changes are associated with the trait for which the mutation is named. In some cases the gene is obviously pleiotropic, very different structures being affected such as bristles and wings or legs and antennae (Caspari, 1952).

When different mutations occur at a single locus forming a multiple allelic series, the genes may usually be arranged in order of dominance of the traits or, if blending occurs in the compounds, in order of increasing effects. This suggests a single type of material differing quantitatively in the different alleles. Many genes, however, are not single physiological units or types of substance but appear to consist of different elements governing different traits. In such cases the gene or locus is said to be complex. The unity of complex genes consists merely in the fact that their hypothetical elements segregate in meiosis without recombing. A pair of complex genes may exhibit complete or partial complementarity in their compound if dominance of their elements is in opposite directions. Complementarity is illustrated by the blood groups when alleles determine the presence of different antigens. Antigens are dominant, antibodies recessive. The compound shows both antigens but neither antibody. Thus in the Landsteiner A, B, O series, the individual of group AB has antigens A and B but antibodies against neither. He is a quadruple dominant heterozygous for these two antigens and also for the two recessive antibodies, anti-B and anti-A. He is also homozygous recessive for anti-O. These serological traits characterizing blood type AB are determined by a single pair of complex allelic genes designated AB.

The present paper reports comparable eye color genes in two species of chalcidoid wasps belonging to different subfamilies of the Pteromalidae. In each species a complex locus has been found with two mutant complementary alleles which are similar in effect. This similarity together with complementarity, indicates homology between the two species. There is added a discussion of similarities and differences in appearance and reactions characterizing the species.

MORMONIELLA

The R alleles

In the chalcidoid wasp *Mormoniella vitripennis* (Walker) (*Pteromalidae: Pteromalinae*), a two millimeter ± parasite of fly pupae, a series of mutant alleles for eye color has been demonstrated, all recessive to wild-type brown. Five of these alleles resulting from mutations following x-radiation of females may be mentioned—(1) dahlia (*R*da), a dark red found by Gladys Friedler, (2) vermilion (*R*vm), a bright red with a prominent black fleck found by Marion Kayhart, (3) scarlet-DR (*R*st-DR), a bright red with minute black fleck found by D. T. Ray,

(4) oyster-white-DR (*R*oy-DR), lacking pigment altogether, a transparent showing the black color of the underlying integument, found by D. T. Ray, (5) oyster-white-NH (*R*oy-NH), phenotypically similar to oyster-white-DR, found by Nellie Harris among progeny of x-rayed scarlet-DR females. Alleles (1)–(4) resulted from x-radiation of wild-type females. Genetic data, some of which have been published (Whiting, 1951), are consistent with a concept of two gene elements each of which has undergone different mutant changes. Thus wild-type brown is + +, showing the two dominant gene elements, dahlia is *da* +, vermilion + *vm*, scarlet-DR + *st*, oyster-white-DR *oy* +, oyster-white-NH *oy st*. Lower case letters are used for these eye color traits because all are recessive to wild type whatever may be their relationships to each other in the compounds.

For the five mutant alleles there have been obtained the expected five types of haploid males, five homozygous females, five heterozygous females with complete dominance of wild-type brown and ten compound females showing dominance relationships as given in table 1. Each heterozygous female and each compound female produces the two expected types of haploid sons from unfertilized eggs. There are no diploid males such as have been found in the ichneumonoid wasp

TABLE 1. Mormoniella. *Phenotypes and dominance relationships for the ten different females possible as compounds for five alleles formed by two mutations (to* da *and to* oy*) in one hypothetical gene element and by two mutations (to* vm *and to* st*) in another*

Compound females	Hypothetical gene elements	Phenotypes	Dominance relationships
dahlia / vermilion	da + / + vm	brown	complete complementarity
dahlia / scarlet-DR	da + / + st	brown	complete complementarity
dahlia / oyster-DR	da + / oy +	bright red	partial dominance
dahlia / oyster-NH	da + / oy st	bright red	partial dominance
vermilion / scarlet-DR	+ vm / + st	light vermilion	blending
vermilion / oyster-DR	+ vm / oy +	brown	complete complementarity
vermilion / oyster-NH	+ vm / oy st	light vermilion	blending
scarlet-DR / oyster-DR	+ st / oy +	brown	complete complementarity
scarlet-DR / oyster-NH	+ st / oy st	scarlet	complete dominance
oyster-DR / oyster-NH	oy + / oy st	oyster-white	homozygous recessive for the masking oyster element

Habrobracon. All fertilized eggs are female-producing.

Oyster-white-DR and oyster-white-NH are isoalleles being of similar phenotype, lacking pigment altogether. They show no complementarity in the compound between them but their difference is demonstrated in their compounds with vermilion and with scarlet (table 1).

This complex locus is designated the R-locus, for David T. Ray who by x-radiation in 1948 obtained the first eye-color mutations in Mormoniella, the completely complementary alleles, oyster-white-DR and scarlet-DR. Many alleles in addition to those listed in table 1 have since been obtained, the dominance relationships of which are very complex and not yet fully analyzed. Partial complementarity is shown by some in which the compound female has eye color much darker than either pure type but brighter than wild type. The nature of the R-locus will probably prove to be much more complex than indicated in table 1.

Gene symbols and gene elements

It is customary in genetics literature to express multiple allelism by superscribing the symbols for the traits to the symbol for the locus. Thus the symbols *da, vm, st-DR, oy-DR,* and *oy-NH* might be superscribed to *R*. Because small superscripts are difficult to see and because they complicate matters for the typesetter, the writer has suggested (Whiting, 1950a) using "full-sized letters only, designating the locus by italics, the multiple allelic traits by roman," for example *R*da, *R*vm, *R*st-DR, etc. If a single allelic series is under consideration the symbol for the locus may be omitted and the symbols for the traits italicized. Thus the formulae may be made less cumbersome.

The allelic genes of a complex locus behave in a manner similar to those of a simple locus. This means that the gene elements segregate in meiosis as a group without recombining with the gene elements in the allele. Not more than two types of eggs are therefore produced by any one female. Different gene elements may be said to be ***alternative*** to each other if they never occupy the same gene. Thus the element *oy* is alternative to *da* since these two are not found in the same gene. However *oy* is not alternative to *st* because a single gene, *R*oy-NH, may possess both of these elements, *oy st*. Non-alternative recessive gene elements in different allelic genes give a complementary effect in the compound, because each has its dominant alternative in the allelic gene, as for example $\frac{oy \quad +}{+ \quad st}$, which is double dominant, wild type.

Interaction of different loci

Several eye-color genes showing segregation independent of the *R* locus have been found in Mormoniella. Some of these are phenotypically similar to scarlet and oyster in the *R* series, representing the loss of almost all "black" pigment and of all pigment respectively. Two dark reds have been reported,—garnet, *ga,* and tomato, *to*. The latter is of low fecundity and poor viability. In color it is rather dull and uniform, lacking any dark or "black" fleck. Garnet is a brownish red possessing a considerable amount of "black" pigment. It has been shown (Whiting, 1950b) that garnet and tomato segregate independently of each other, as well as of the *R* series, and together, *ga to,* they give an orange color, distinctly different from either acting alone.

Table 2 shows the various interactions of garnet and tomato with members of the *R* series. Because oyster removes all pigment from the eyes, it masks factors causing color differences. The dark color seen in oyster was at first thought to be due to some "black" pigment lying deep in the eye itself (Whiting, 1951) similar to that causing the black fleck in vermilion. Sectioned material fails to reveal any such pigment in the oyster eye. The dark color

TABLE 2. Mormoniella. *Interaction of eye color genes garnet*, ga, *and tomato*, to, *with alleles in the* R *series*

	+ +	ga +	+ to	ga to
R alleles				
+ +	brown	dark red	dull red	orange
da +	dark red	bright red	dull orange	dull orange
+ vm	bright red dark fleck	scarlet	bright orange	bright orange
+ st	scarlet	scarlet	bright orange	bright orange
oy +	oyster	oyster	oyster	oyster
oy st	oyster	oyster	oyster	oyster

is due to transparency revealing the black underlying integument.

Garnet, like dahlia, causes reduction in amount of "black" pigment from the wild type. Their effect is cumulative making the double recessive bright red, clearly distinguishable from the single recessives and somewhat darker than scarlet. Garnet is masked by scarlet and reduces the black pigment in vermilion so that vermilion garnet looks like scarlet.

The reaction of tomato with vermilion and with scarlet causes a bright orange color, somewhat brighter than that of garnet tomato. The triple recessives, vermilion garnet tomato and scarlet garnet tomato, *vm ga to* and *st ga to*, are also bright orange. This indicates that enough pigment is left in the eye to cover the black integument. In contrast, the dull orange reaction with dahlia is due to the fact that pigment reduction is extreme enough to permit the black color of the underlying integument to show through, giving a grayish cast and suggesting an approach toward oyster. Reactions with tomato then serve to emphasize similarity between the alternatives dahlia and oyster and their contrast to the alternatives vermilion and scarlet. Tomato, having no "black" pigment, removes "black" from dahlia, revealing that there is little "red" left in contrast to vermilion tomato and scarlet tomato. A qualitative difference also is to be noted—tomato in combination with any other red gives orange. This may be seen in garnet tomato in contrast to dahlia garnet, the latter giving a bright red approaching scarlet.

Pachycrepoideus and Mormoniella

Investigations have been carried out with *Pachycrepoideus dubius* Ashmead (*Pteromalidae: Sphegigasterinae*), a chalcidoid wasp somewhat similar in form to Mormoniella but smaller in size and bluer in color. Like Mormoniella it is arrhenotokous, producing males from unfertilized eggs, females from fertilized. No biparental (diploid) males have been found. Both species parasitize pupae of Diptera, the females ovipositing through the puparium in the space surrounding the pupa. Mormoniella attacks the larger species, the calyptrates such as blow flies and house flies. Its larvae are not cannibalistic and many individuals, 100 ±, may be obtained from a single host. Pachycrepoideus attacks the smaller species, the acalyptrates such as Drosophila and Piophila, the cheese-skipper. Its larvae are cannibalistic and usually not more than a single individual is able to develop in one puparium, although more than one egg may be laid on the pupa. Size of the emerging wasp is determined within limits by the amount of food material available,

whether that food consists of the host pupa or a less fortunate sibling larva. Wasps emerging from large puparia are therefore in general of rather uniform size and larger than those from smaller puparia. However a few very small specimens occur. These are fortunate enough to have escaped their better-fed "twin" from the same puparium, which will show two exit holes of different size.

In addition to the large numbers to be obtained from single puparia, and its somewhat larger size, Mormoniella has many other advantages over Pachycrepoideus as material for genetics studies. The males are flightless, brachypterous, and the females, although well able to fly, may safely be placed upon a table where they will run about and may easily be covered with glass vials in order to distribute them into separate cultures. Both males and females of Pachycrepoideus take readily to flight. It is necessary to keep them under ether at all times while handling or they disappear instantaneously. In both species matings normally occur immediately or shortly after emergence from the host puparia. If males are not present, the females soon lose their instinct for mating so that male broods result. This occurs sooner in Pachycrepoideus than in Mormoniella and, similarly, the mating instinct of the male is more ephemeral in the former than in the latter.

With Mormoniella, isolation of virgin females is very easy. The large host puparia are broken open with a needle and the parasite pupae dumped out on a dark card. The sexes may readily be separated under a binocular by noting the differentiating genitalia of the female. With Pachycrepoideus it is more convenient to remove the wasps frequently from the culture vials by use of ether and to count females as virgins only when no males are present. Because of these difficulties in obtaining virgin females and in getting daughters from virgins if they are not immediately set with males, the experiments were planned, whenever possible, to permit random matings in mass cultures.

To determine sex of adults in Pachycrepoideus, genitalia must be carefully noted, a rather tedious task when large numbers are being classified. There are no conspicuous secondary sex traits as there are in Mormoniella such as short wings of the males, their lighter colored antennae and legs and brighter body color in general.

Development is more rapid in Mormoniella than in Pachycrepoideus. At 30° C. the period from setting the parents until eclosion of the offspring is 10–12 days in the former, 14–16 days in the latter.

Genetics and biology of Mormoniella, together with technical methods for rearing are being discussed in detail elsewhere.

Pachycrepoideus

Preliminary tests and technique

Pachycrepoideus stock was supplied by Dr. James C. King of the Biological Laboratories at Cold Spring Harbor, Long Island, N. Y., in April 1951. The parasites had been infesting his cages of Drosophila used in population genetics studies. In preliminary tests stock females exposed to males produced 955 daughters and 527 sons. Unmated females produced 312 sons and no daughters.

Tests were made for parasitism of pupae of some rather small-sized calyptrate flies such as Musca, Lucilia and some parasitic flies from mud-dauber wasp nests. Over 150 flies eclosed and about 75 fly pupae died in puparia, probably because of being stung by the wasps. Five dead parasite larvae were found in separate puparia. Fifteen adults emerged, twelve females and three males, two of the females emerging from a single puparium. The yield of parasites from these hosts was therefore very poor in number but the adults that did emerge were of good size.

Large numbers of the wasps may be

reared in mass cultures of Drosophila. However, it is very time-consuming to recover them from the culture bottles and to separate them from the flies. A technique was therefore devised for rearing the wasps in shell vials. Large species of Drosophila, *D. virilis* and *D. funebris*, were used as hosts since this insured better growth of the parasites.

Mass cultures of Drosophila were reared in glass storage jars five inches high by eight inches in diameter. The culture medium, corn meal and molasses well-seeded with yeast, was kept very moist. A muslin cloth was tied tightly over the top and secured about the rim of the jar to prevent contamination by stray parasites. The glass cover placed loosely over the muslin held humidity high to ensure oviposition and later to induce the maggots to crawl high up the side for pupation. The freshly-formed pupae were scraped off the glass with a scalpel and distributed with a camel's hair brush over strips of paper which had previously been wet in agar suspension. These strips were about one inch in diameter and four inches long. When dry, the agar served to fasten the puparia firmly. These strips were placed in large shell vials, one to several in each according to the numbers of parasites serving as parents for the next generation. The vials were stoppered with cotton plugs covered with cheese cloth.

The parasite females moved about between the strips stinging the pupae, feeding on their juices and ovipositing. When it was desired to transfer the wasps, ether vapor was introduced into the vial so that they would fall to the bottom. The contents of the vial were then poured out onto a card, the strips with the pupae adhering were replaced in the vial and the parent wasps were set with fresh pupae in a second vial. When offspring eclosed, they were similarly etherized to separate them from the strips which were replaced in the vials for subsequent eclosions. It was found convenient to cut the ends of the strips at an angle of about 45° so that a point rather than an edge would be in contact with the bottom of the vial. Thus guillotining of etherized wasps was avoided when the strips were dropped into a vial.

Crandall (1939) has published an excellent and very comprehensive account of the biology of this insect.

X-ray tests

Freshly eclosed stock females of Pachycrepoideus exposed to males and x-rayed with 2500 roentgen units at the Marine Biological Laboratory, Woods Hole, Massachusetts, produced 1141 daughters and 1433 sons. Among the sons there were 14 mutants with bright eye colors—one garnet, two tomato, five scarlet and six oyster-white, giving a mutation rate of one per cent (95% confidence limits = 0.54—1.64%) which is comparable to that obtained in Mormoniella. Daughters of x-rayed females, similarly x-rayed with 2500 r, produced 278 daughters and 952 sons. Among the latter there were 22 with bright eyes—four peach, six scarlet and twelve oyster-white. These were in part new mutants but in part segregants from mothers heterozygous for mutant genes induced in their mothers by the previous raying. This condition was indicated by the fact that the four peach occurred in a single culture as did also three of the scarlet and four of the oyster. Mutation rate cannot, therefore, be calculated for this group because of replication.

If a female from untreated stock is irradiated with 2500 r or over, replication of new mutations is not to be expected. This is because of sterility ensuing after her eggs treated as oöcytes have been laid. Many treated females were transferred to a second vial but no offspring were obtained. When untreated females were transferred, it was possible to obtain further offspring.

Heredity of mutant traits

Many virgin stock females of Pachycrepoideus exposed to the mutant males produced no daughters, demonstrating the difficulty of getting the desired matings. There were counted 3115 sons. Matings were obtained from scarlet mutants resulting in 36 daughters, 258 sons; from tomato mutants giving 65 daughters, 87 sons; from peach mutants giving four daughters, 45 sons; and from oyster mutants giving 76 daughters, 176 sons. All daughters were wild-type brown like their brothers.

A few very dark red "mutants" from the x-rayed cultures sired 105 daughters with 161 brothers. From the "heterozygous" daughters there were obtained 399 females and 226 males, all wild-type brown. Two females with very dark red eyes occurring in later cultures produced 27 wild-type sons. These very dark red therefore appear to be merely a fluctuant type. The same occurs also in Mormoniella but it has been shown there that some very dark reds are hereditary.

Descendents of the four successfully mated mutant types showed segregants as expected. Mutant-type females were obtained which had mated with wild-type or mutant-type "brothers." The offspring are shown in table 3. Although there are 1588 wild-type daughters from these mutant-type females indicating mating with wild-type males, there are no wild-type sons. This shows that diploid biparental males such as occur in Habrobracon are lacking in Pachycrepoideus as they have been shown to be lacking in Mormoniella.

TABLE 3. Pachycrepoideus. *Progenies from mutant-type females which had been exposed to to their mutant-type and wild-type "brothers"*

Mutant types of females	Daughters		Sons
	Wild-type	Mutant-type	Mutant-type
Scarlet	326	333	301
Tomato	356	244	450
Peach	29	15	16
Oyster	877	351	830
Totals	1588	943	1597

TABLE 4. Pachycrepoideus. F_2 *from mutant-type females crossed with wild-type males*

F_1		F_2			
		Females		Males	
Wild-type females	Mutant-type males	Wild-type	Mutant-type	Wild-type	Mutant-type
st/+	st	67	70	156	108
to/+	to	156	134	284	253
pe/+	pe	63	49	29	16
oy/+	oy	95	89	70	56

Wild-type daughters of mutant-type females were set. These had mated to mutant-type "brothers." Data are given in table 4. In these cultures the eye colors are expected in 1 : 1 ratio in both sexes. Deviations not due to chance may be caused by linked lethals or by lower viability of the mutant types.

Mutant stock cultures were derived and maintained in large wide-mouthed culture jars of Drosophila covered tightly with muslin which served both to avoid contamination and to reduce excessive humidity. Parasitized pupae were removed from these jars, virgin females were obtained and crosses made. Offspring and descendents are shown in table 5.

It was expected that tomato and peach might be alleles. There is no dark pigment in either and no dark fleck. Both appear rather dull uniform red resembling tomato in Mormoniella but peach is lighter. The traits overlap somewhat, a freshly eclosed tomato being as light as an old peach. Their allelism is shown by the four daughters from a peach female mated with a tomato male (table 5, no. 1). These were tomato, as dark as their tomato brothers, showing dominance of tomato in the compound females. No second generation was obtained.

Reciprocal crosses were made between tomato and oyster and oyster females were crossed to peach males (table 5, nos. 2–4). The F_2 data indicate independent segregation of the two loci, 1 : 1 : 2 ratio being ex-

pected, with masking effect of oyster as in Mormoniella.

When scarlet females were crossed to tomato males (table 5, no. 5) independent segregation was indicated. A single male occurred with eyes mosaic for scarlet and tomato, probably from an unfertilized binucleate egg. Scarlet tomato is orange differing from tomato in having much less red pigment. Actually it looks darker than tomato because of translucency, the black color of the underlying integument showing through. Scarlet tomato stock was derived from scarlet females heterozygous for tomato (st/st + /to). Scarlet tomato females were crossed to tomato males (table 5, no. 6) giving tomato females, which were much redder than the orange of their scarlet tomato brothers. The presence of the tomato gene, whether or not scarlet is present, dulls the red, making it readily separable from scarlet.

Scarlet females exposed to peach and to scarlet peach males (table 5, no. 7) produced both wild-type and scarlet daughters showing that they had mated with both types of males. Offspring of the wild-type daughters produced the expected classes, but separation of peach from scarlet peach was uncertain. Red pigment is much reduced in both. Scarlet peach appears somewhat blacker than peach because of greater translucency. When they are placed in alcohol they become opaque and light pink, the scarlet peach being almost white.

Stock scarlet females crossed to oyster males (table 5, no. 8) produced wild-type daughters and scarlet sons as expected. Males in the second generation were of two classes only showing allelism of scarlet and oyster as in Mormoniella. The reciprocal cross (table 5, no. 9a) gave comparable results, no wild-type male recombinants appearing in F_2. Wild-type females selected from this and a later generation (table 5, no. 9b) again gave no wild-type recombinant males. The 221 scarlet and 443 oyster daughters sired by scarlet and oyster fathers totalled 664.

TABLE 5. Pachycrepoideus. *Progenies and descendants from crossing mutant types*

Cross no.	P ♀ × P ♂	F_1 ♀ ♀	F_1 ♂ ♂	F_2 ♀ ♀	F_2 ♂ ♂
1	to × pe	to 4	to 12		
2	to × oy	+	to	+42, to 24	+4, to 9, oy 8
3	oy × to	+ 221	oy 291	+389, oy 407	+157, to 125, oy 242
4	oy × pe	+26	oy 58	+92, oy 106	+21, pe 18, oy 40
2–4		247	349	523 537	182 152 290
5	st × to	+32	st 68	+195, st 175	+63, st 71, to 64, st to 39, st-to mosaic 1
6	st to × to	to 30	st to 10		
7	st × pe & st pe	+13 st 10	st 24	+18, st 18	+9, st 2, pe? 11 st pe? 5
8	st × oy	+21	st 21	+70, st 63	st 41, oy 33
9a	oy × st & oy	+263 oy 60	oy 321	+141, oy 163	st 184, oy 184
9b	+F_2 & +F_3 ♀ ♀ from 9a			+671, st 221 oy 443	st 390, oy 411
8–9		+284	st & oy 342	+882, st & oy 830	st 615, oy 628
3–9		dom. 606	rec. 793		

close to the 671 wild type as expected. Offspring of wild-type females compound for scarlet and oyster (table 5, nos. 8–9) total females wild type 882, scarlet and oyster 830, males scarlet 615, oyster 628, showing complementary allelism of the genes as in Mormoniella.

In this series of experiments (table 5) offspring of recessive females by dominant males total 606 dominant females and 793 recessive males. The terms recessive and dominant refer to separate genes in crosses 2–7. In crosses 8–9 these terms refer to hypothetical gene elements. Thus the gene scarlet has an element dominant over its alternative in the gene oyster and likewise a second element recessive to its alternative in the gene oyster. Complementarity results because dominance of these elements is in opposite directions.

Daughters from recessive females crossed to dominant males (tables 3 and 5) total 2194 with the paternal dominant trait. There were 2390 sons, all recessive. It is therefore highly improbable that diploid biparental males occur in this species.

Discussion

The word homology has different meanings. To the paleontologist and the morphologist it means one thing, to the cytogeneticist it means another. The vertebrate eye has preserved its essential similarity during the course of evolution, although the genic basis must have become altered in large part at least. There has been a continuity of the organ maintained by selective adaptation so that the eye of a man is homologous to that of a fish. Homology in this sense does not refer to chromosome loci.

Striking similarities in color pattern occur between species of insects belonging to different orders. Syrphid and asilid flies may be pinned in insect boxes alongside their hymenopterous models. For the syrphids the resemblance is "protective." As the story goes, a bird will not eat a fly that resembles a wasp fearing it may be stung. For the predaceous asilids, however, the resemblance is "aggressive." The hairy fly marked with black, yellow and orange, sneaks up to victimize the similarly marked bumblebee which innocently thinks it to be one of its own kind. In real life, however, the asilid does not feed upon bumblebees which it resembles but upon smaller insects which it does not resemble. It stands alert with "all its eyes open" watching for its passing prey and looking not at all like a busy bee buzzing about flowers. Several years ago on a trip through the Selkirk Mountains the writer collected not only flies and bees marked with orange, black and yellow but also beetles and caterpillars with similar color pattern. The result was *reductio ad absurdum* for the mimicry hypothesis in this particular case. The traits, however, are strikingly similar.

What is meant when the question is asked—Are these traits homologous? It is not supposed that all the ancestors of these diverse groups had the orange stripe or even the gene for the orange stripe, but that there had always been present a certain chromosome locus that could mutate so as to produce the orange stripe. Are such trivial characters then homologous in this sense, that they are subtended by gene loci common to different insect orders? Historically considered, the idea may seem far-fetched and for some mimetic traits it is probable that they are not dependent upon homologous chromosome loci. The resemblance if not fortuitous is a matter of adaptation. Ford (1953) cites the case of two longicorn bettles, mimics of an Australian wasp. In one of them the pattern, brown with a transverse black band, is copied on the elytra, but these are short in the other species and the mimetic coloring is on the exposed abdomen. Also the red marks of mimetic females of the butterfly *Papilio polytes* are different chemically from the red of their models of the genus Polydorus. Such resemblances are probably not due to the

possession of common ancestral chromosome loci.

If species are so closely related that hybrids may be produced, homology can be tested by crossing mutant types with similar traits. Convincing evidence of homology is also furnished for more distantly related species by comparison of linkage maps and especially (in Drosophila) of salivary chromosome maps.

Studies of natural mosaics, feeding and injection of larval extracts and transplantation of eye disks indicate biochemical similarities of pigments suggesting homology for nonautonomous differences (Beadle, Anderson and Maxwell, 1938). Spectrophotometric analyses of pigments indicate what substances are not and what may be determined by genetically homologous loci for mutant types in different orders of insects (Villee, 1947).

In diverse orders of mammals there occur the recessive changes in hair color from dense pigmentation, D, to slatey dilute, d; from black, B, to brown, b; from agouti, A, to uniform non-agouti, a; and from full color, C, to pink-eyed albinism, c. The last two loci, A and C, show intermediate alleles in several species. If such changes were not homologous, if mutation in non-homologous loci might produce slatey dilute, brown, non-agouti or albino in different species, then it might be expected that non-homologous mutant types showing the same phenotype would occur in the same species. In this case two different albino mice, for example, might be found upon crossing to produce double-dominant colored offspring. The classic cases of recessive whites in poultry or in peas giving colored F_1 and a 9:7 ratio in F_2 indicate that white birds or white flowers of different species may be white for different reasons (non-homologous loci). The failure as yet to find a comparable situation with respect to any of the four recessives mentioned in mammals, despite extensive breeding tests, indicates that these mutant types are homologous from group to group.

White eye mutants have occurred in several species of Diptera, ranging from the least specialized (Psychoda) to the most (Calliphora) and in the Hymenoptera, the ichneumonoid Habrobracon, the aculeate honey bee and the chalcidoids Mormoniella and Pachycrepoideus. In Habrobracon white (wh) and ivory (o^i) are indistinguishable phenotypically but the latter is non-autonomous in mosaics. In the honey bee three whites have recently been reported (Rothenbuhler, Gowen and Park, 1953); ivory (i), cream (cr) and snow (s), phenotypically indistinguishable, but like white and ivory in Habrobracon giving wild-type black on crossing and segregating independently in the F_2 males. Obviously then there is no basis for assuming genetic homology between white eye mutants in different species of insects unless other evidence is at hand.

Evidence for genetic homology may become available from the study of complex loci with comparable alleles in different species. The Rhesus locus for blood groups in man has a long series of alleles determined by two to several alternative differences in each of three subloci designated D, C and E. It is also possible that the antigenic factor called f may be determined, not by any one of these gene elements in itself but by the relative position of two of them, "position effect" (Jones, Steinberg, Allen and Diamond, 1953). This complex Rhesus series in man may have its homologue in the monkey which is thus far reported as Rhesus positive. Homology of the chromosome locus between man and monkey might be indicated if different Rh factors were found in the latter—D, C and E, and Hr factors—d, c and e, and if their various combinations could be shown to form an allelic series. Breeding test to demonstrate this would be technically very difficult.

Many of the color patterns in different species of grouse locusts, Tetrigidae, are strikingly similar (Nabours 1929, Nabours, Larson and Hartwig, 1933). For

the most part these act as dominants over the "wild type" which is a relatively uniform brownish gray. The patterns affect the pronotum or the hind femora. There may be a white spot on the femora; the femora may be marked with reddish brown or with yellow and these marks may be restricted or extended; the pronotum may be all white, or it may have white or black restricted to the anterior region or to a spot; there may be a red or a yellow median longitudinal band on the pronotum. These simple traits may act as a series of multiple complementary alleles. In the compounds the traits are superposed so that both show if different regions are affected or they may conflict or one may mask the other in the same region. Rarely a recombination may take place and two or more of the traits may be combined, the combination acting thereafter as another allele in the series. The traits thus combined may be unaltered or there may be alteration so that the new allele gives a different effect from that of the compound in which it originated.

In *Paratettix texanus* there are twenty-five or more of these simple traits and their combinations, acting as a series of alleles with only three crossovers in 110,-572 gametes tested. One of these crossovers (between a mahogany spot, I, and a pale yellow band, S) involves modification. This "crossover IS position-effect mutation" acted as a new allele, Sm, in the series, failing to shown crossing-over in 13,786 gametes tested. Another pattern factor, Hm, showed crossing-over with this series three times in 7278 gametes tested and two other factors segregated independently. In *Acrydium arenosum* the factors for the patterns are distributed along the different pairs of autosomes and may be readily recombined. Intermediate conditions with very close linkage or allelism of some traits are found in other species such as *Apotettix eurycephalus*.

It appears that complex genes are built up by tying together simpler genes, which then come to constitute gene elements. This tying together may be done by localization of chiasmata or by inversions (Darlington and Mather, 1949). Genes which are thus tied together may continue to exert their original effects unmodified, or there may be position effects so-called, with unexpected or modified traits appearing. In the latter case the crossing-over is one type of mutation. Gene elements within the same complex gene may be independent of each other in mutation rate or the mutation rate of one may be modified according to the alternative of a second element with which it is combined as in the R locus of maize (Stadler, 1951). The lozenge-eye locus of Drosophila has three subloci which may mutate to any one of a series of recessives causing the lozenge effect. Any homozygote is lozenge, but also any compound whether the difference involves alternatives at the same sublocus or at different subloci. In the latter case it is shown that the wild-type gene elements are not dominant unless they are associated in the same gene (Green and Green, 1949).

What then is the difference between a gene complex and a complex gene? This problem is similar to that of races vs. species. Races evolve into species and gene complexes may be evolving into complex genes. The definition is, therefore, arbitrary and a matter of convenience. It may be suggested that we have a pair of pleiotropic genes rather than two groups of genes if the traits fail to recombine in meiosis or if recombination is infrequent and is accompanied by so-called position effects.

It is highly probable that the complementary alleles oyster and scarlet in Pachycrepoideus indicate an R series, a chromosome locus genetically homologous with the R locus of Mormoniella. However, if complex genes are built up from simpler genes, as seems highly probable, it is possible that these alleles may have been derived from different genes in the two species.

With the technical methods now available it would not be difficult to pursue

further investigations that might more definitely indicate homologies in loci of these two species, as also of certain other species of Chalcidoidea.

Summary

Similarities and differences are discussed between two species of chalcidoid wasps, *Mormoniella vitripennis* (Walker) and *Pachycrepoideus dubius* Ashmead, belonging to the same family but to different subfamilies. Both parasitize pupae of Diptera, Morminiella parasitizing large species, Pachycrepoideus parasitizing small. In both species unmated females produce males parthenogenetically. All fertilized eggs are female-producing. Method of sex determination appears to be different from that of the ichneumonoid Habrobracon. A series of x-ray and spontaneous multiple mutant alleles, the "R-locus" series, changing wild-type brown eye color to the recessives oyster white and various reds, has been found in Mormoniella. Many at least of their interactions may be explained by postulating two gene elements. Different mutations in the same element are characterized by blending of the traits or partial dominance of one over the other in the compound females. If the mutations are in different elements, the compound shows the double dominant wild phenotype. Unmated heterozygous and compound females produce the two expected types of haploid males in equal numbers.

X-radiation of Pachycrepoideus females has produced similar eye-color mutations. Two of these, scarlet and oyster-white, are counterparts of two in the R-locus of Mormoniella, similar in appearance and acting as complementary alleles, giving wild type in the compound females. A second locus in each species has mutated to a dull dark red, tomato, which gives an orange color in combination with scarlet. It is postulated that the R-locus is homologous in the two species. Homology of tomato is also possible but less certain.

The meaning of homology is discussed. One type of homology refers to persistence of a structure, such as the vertebrate eye, throughout the course of evolution. We are not here concerned with the genic basis which may undergo considerable change. The similarity is maintained by natural selection and adaptation.

Another type of homology refers to chromosome loci. This does not mean that the similar traits or the genes subtending the traits have persisted during the evolution of the different groups, but rather that "wild type" chromosome loci which may mutate to produce such traits have been transmitted from a common ancestor.

"Mimetic" traits of color pattern in different insects are considered. Similarity may be due to selective advantage and genetic basis may or may not be homologous.

Failure to find different genes causing similar color phenotypes within the same species of mammals indicates that when these colors occur in different species they are homologous. If different genes cause the same phenotypes within the same species as in white eye mutants of Diptera and Hymenoptera, interspecific homologies cannot be assumed without further evidence.

Homology of similar mutant types occurring in closely related species may be proved by species crosses or, when this is impossible, by comparison of linkage and salivary chromosome maps.

Evidence for genetic homology may be gained from a study of complex loci in different species. If it were possible to prove the existence of an allelic series in the Rhesus monkey similar to the Rhesus blood group series in man, this would be good evidence for homology of the chromosome locus.

In different genera of grouse locusts, Tetrigidae, color patterns acting as dominants over a uniform recessive occur. These patterns are complementary in crosses so that the heterozygotes tend to show them all. In some species many of

these pattern factors form a multiple allelic series. The simpler patterns may be combined into more complex by crossing-over and the new combination forms a new allele in the series. In *Acrydium arenosum* the pattern factors have different chromosome loci. The bearing of these facts upon the nature and evolution of complex genes and upon genic homologies is discussed.

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INTERFERTILE SIBLING SPECIES IN THE *WILLISTONI* GROUP OF DROSOPHILA

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Among the wild species of *Drosophila* found in neotropical America, the *willistoni* group has attracted the most attention. *Drosophila willistoni* itself has a natural distribution which extends from Florida to Argentina (Townsend, 1952; Pavan, 1952) and over much of this range it is the most abundant species of the genus in collections. The overwhelming biological success of this species appears to be correlated with the occurrence in its chromosomes of an extraordinary amount of naturally-occurring polymorphism due to the presence of inverted sections (da Cunha, Burla and Dobzhansky, 1950). Interest in this species is furthermore greatly enhanced by the fact that, within its general geographical range, there co-exist at least three "sibling species" which cannot be easily separated from *D. willistoni* on morphological grounds (Burla *et al.*, 1949). Isolation between these four entities is nonetheless absolute, both in nature and in the laboratory.

Perhaps because of preoccupation with the many problems presented by the existence of this tetrad of morphologically very close species of which *D. willistoni* is a part, the other neotropical species belonging to the *willistoni* group of the genus have not been intensively studied. These more distantly related species include *D. fumipennis, D. capricorni, D. nebulosa, D. sucinea* and *D. bocainensis*. Except for the work of Pavan (1946) and da Cunha, Brncic and Salzano (1953) on *D. nebulosa,* no quantitative studies of the population genetics of these forms have been made.

In order to begin the closing of this gap in the knowledge of this most significant group of flies and in order to provide a basis for comparisons of evolutionary patterns within the *willistoni* group as a whole, the present study was undertaken. Quantitative data on populations of *Drosophila bocainensis* Pavan and da Cunha 1947 are presented. Within this older taxonomic designation, it will be shown that there exist three reproductively isolated Mendelian populations. Evidence for the specific distinctness of these three entities is given. Two of the three, although they are partially sympatric and essentially indistinguishable morphologically, show no crossing under natural conditions. This isolation, however, breaks down strikingly in the laboratory; under these conditions, the two species hybridize freely and produce abundant fertile offspring.

MATERIAL AND METHODS

Specimens of *Drosophila* were collected by the method of Pavan and da Cunha (1947) from a number of localities in the states of São Paulo and Rio Grande do Sul, Brazil during the period July to November, 1951. Each wild female which conformed to the morphological description of *Drosophila bocainensis* Pavan and da Cunha 1947 was placed in an individual culture tube containing banana-agar food inoculated with fresh Fleischmann's yeast. Cytological preparations of the salivary gland chromosomes of F_1 larvae from each wild female were made by the usual aceto-orcein technique.

DESCRIPTIONS OF SPECIES

In the body of this paper, quantitative cytogenetic evidence is adduced to show that three specific entities have previously

been included within the designation *Drosophila bocainensis* Pavan and da Cunha 1947. Only one of these three (*D. bocainoides* sp. nov.) is easily separable morphologically; the other two are very closely related sibling species. For one of the sibling species, the designation *Drosophila bocainensis* Pavan and da Cunha 1947 will be retained; the other will be described as *Drosophila parabocainensis* sp. nov. No single morphological character, or combination of characters, has been found whereby individual members of the two sibling species may be reliably separated. Statistical differences in certain structures, notably the male genitalia and in length of larval-pupal life are apparent and will be the object of further study. The species may be separated quickly and reliably by examination of the salivary gland chromosomes of F_1 larvae, as will be shown later. It may be convenient to refer to these three species as comprising the *bocainensis* subgroup of the *willistoni* group of Drosophila.

The type material of *Drosophila bocainensis* Pavan and da Cunha 1947 was collected in February, 1944 at a remote fazenda called Campos da Bocaina in the extreme northeastern corner of the State of São Paulo, Brazil. This fazenda lies in a tongue of the state which projects eastward into the State of Rio de Janeiro and is approximately equidistant (30 km.) from Quéluz, on the railroad Central do Brazil, and the town of Bananal.

As the laboratory strain from which this species was described is no longer in existence, it is not possible to assign the type material with certainty to one or the other of the species now recognized cytologically, nor has it been possible to make further collections from the type locality. Campos da Bocaina, however, lies in the coastal rainforest of the Serra do Mar, 270 km. (145 miles) north and east of Vila Atlântica, an area south of Santos from which extensive collections of Drosophila have been made (see Dobzhansky and Pavan, 1950). Both locations are closely similar ecologically and lie in the same mountain range. At Vila Atlântica, only one of the two sibling species has been found; this same sibling has likewise been found abundantly at two other locations in eastern São Paulo from which *bocainensis*-like flies have been analyzed cytologically. These locations are the Horto Florestal in the Serra Cantareira, near the city of São Paulo, and Mogí das Cruzes.

For the above reasons, it seems logical to utilize the original name *Drosophila bocainensis* Pavan and da Cunha to designate the particular member of the sibling species pair found abundantly in coastal São Paulo. As the pinned type material prepared from flies collected at Campos da Bocaina has been lost, a neotype series (five males and five females) was prepared from a laboratory strain of flies derived from the offspring of a single wild female collected at Vila Atlântica in July 1951. These specimens, along with the syntypes of the two new species here described, have been deposited in the personal collection of H. D. Stalker, Washington University, St. Louis. This collection will ultimately be deposited at the American Museum of Natural History, New York. Duplicate series of all three species have been deposited in the Instituto de Zoologia, Museu Paulista, São Paulo, Brazil.

Drosophila bocainensis Pavan and da Cunha

Conforms to the original description of this species by Pavan and da Cunha 1947, pp. 18–20, except for geographical distribution, which is restated here as follows:

Distribution: Brazil: State of São Paulo: Campos da Bocaina (type material, February 1944; types lost; not verifiable cytologically); Vila Atlântica (paratype material, July 1951); Mogí das Cruzes; Horto Florestal, Serra Cantareira, near the city of São Paulo. State of Rio Grande do Sul: Feliz; Ponta Grossa. State of Minas Gerais: Montes Claros.

Drosophila parabocainensis sp. nov.

External characters of imagines

♂. Arista with about 11 branches. Antennae brownish yellow, third segment darker with dense light-colored pile; second joint with

slightly lighter lateral edges. Front uniformly brownish yellow; space between ocellae darker; small hairs curved towards the midline near the point where the frontal lines touch one another; a series of small hairs from the middle orbital to the base of the antenna following the edge of the eyes. Anterior orbitals slightly shorter than the posterior, middle 3/4 the length of posterior. Two prominent oral bristles equally long. Face light brownish yellow. Carina rounded not sulcate. Cheeks approximately the same color as the face, their greatest width about 1/7 the greatest diameter of the eyes. Palpus the same color as the face, slightly darker at apex. No prominent anteriorly directed bristle at apex. Anterior-posterior axis of labellum short, ventral edge not noticeably blackened. Eyes orange-red with short dark pile which is blond ventrally.

Acrostichal hairs in 8 irregular rows. Anterior scutellars divergent. No prescutellars. Mesonotum dull brownish yellow, pleurae somewhat lighter. Anterior sternopleural thin, 1/2 the length of the posterior. Coxae and legs light brownish yellow; prominent apical bristle present on second tibia only, preapicals on all three.

Abdomen: First tergite grayish yellow; second, third and fourth with black (gray or light grayish brown in some specimens) posterior marginal bands, broadened at the midline and at the lateral edges. Anterior marginal band brownish yellow, forming a low crescent spot on each side of the tergite. The posterior tergites are entirely black (gray or light grayish brown in some specimens).

Wings slightly dusky; posterior crossvein usually clouded. Two prominent bristles at the apex of first costal section; third costal section with heavy bristles on its basal 2/5 to 1/2. Costal index 2.8–3.3; 4th vein index 1.6–1.8; 5x index about 1.7. Length of body (fresh specimens) 2.6–3.5 mm.; wing length 2.7–3.0 mm.

♀. A single, prominent, anteriorly-directed bristle at apex of palpus.

Internal characters of imagines

Anterior and posterior Malpighian tubules with free ends. Testes pale yellow with three outer and two inner coils. Spermatheca barely ellipsoidal, moderately chitinized, with a sharp apex. Ventral receptacle folded, resembling a skein of wool.

Other characteristics, relationship and distribution

Eggs: Two filaments with broadened tips, about 1/2 the length of the egg.

Larvae: The larvae do not skip.

Puparia: Yellowish brown; horn very small with about 8 branches.

Chromosomes: Two pairs of V-shaped chromosomes and one pair of rod-shaped chromosomes.

Distribution: Brazil: State of Rio Grande do Sul: Feliz (type material, July 1951); Ponta Grossa; Emboaba; Muitos Capões. State of São Paulo: Pirassununga; Horto Florestal, Serra Cantareira, near the city of São Paulo. State of Minas Gerais: Montes Claros.

Relationship: Belongs to the ***willistoni*** group of the subgenus *Sophophora*. This species cannot be reliably separated on morphological grounds from its sibling species *D. bocainensis*.

Drosophila bocainoides sp. nov.

External characters of imagines

♂. Arista with about 11 branches. Antennae brownish yellow, third joint darker and densely pilose. Front golden yellow; space between the ocellae darker; small hairs curved toward the midline near the point where the frontal lines touch one another; a series of small hairs from the middle orbital to the base of the antenna following the edge of the eyes. Anterior orbitals slightly shorter than the posterior; middle 1/2 the length of posterior. Two prominent oral bristles equally long. Face grayish brown. Carina keel-like and prominent, not sulcate. Cheeks yellow, their greatest width 1/5 greatest diameter of eyes. Palpus the same color as the face, a prominent anteriorly directed bristle at apex. Anterior-posterior axis of labellum forming a long keel, blackened along ventral edge. Eyes red with short, dark pile.

Acrostichal hairs in 8 irregular rows. Anterior scutellars divergent. No prescutellars. Mesonotum dull grayish yellow, pleurae contrastingly dark brownish black. Anterior sternopleural thin, 3/5 the length of posterior. Coxae dark grayish brown; legs grayish yellow; prominent apical bristle present on second tibia only, preapicals on all three.

Abdomen: First tergite grayish brown, second, third and fourth with black posterior marginal bands irregularly narrowed at the midline and broadest at the lateral edges; anterior marginal band yellow. The posterior tergites are entirely black.

Wings slightly dusky, posterior crossveins with a very faint cloud. Two prominent bristles at the apex of the first costal section; third costal section with heavy bristles on its basal 2/5 to 3/5. Costal index 2.8–3.1; 4th vein index 1.4–1.6; 5x index about 1.5. Length of body (fresh specimens) 3.8–4.0 mm.; wing length 3.0–3.2 mm.

♀. Pleurae dull grayish yellow, only slightly darker than mesonotum. Coxae grayish-yellow, the same color as the legs.

Internal characters of imagines

Malpighian tubules not fused at apices; anterior with green or white bulbous swellings

TABLE 1. *Chromosomal evidence for non-interbreeding in nature between two sympatric entities,* Drosophila bocainensis (GROUP 1) *and* Drosophila parabocainensis (GROUP 2). Data based on one F_1 test larva from each of 126 wild females collected at Feliz and Ponta Grossa, Rio Grande do Sul, Brazil, 1951.

	Group 1 *D. bocainensis*	Group 2 *D. parabocainensis*
No. of wild females tested from:		
Feliz	60	42
Ponta Grossa	16	8
Total tested	76	50
Chromosome X:		
pattern at tip of left arm:*		
AA (female test larvae)	53	0
A (male test larvae)	23	0
aa (female test larvae)	0	36
a (male test larvae)	0	14
No. test larvae heterozygous for inversion XR-B	15	0
Chromosome 2:		
No. of test larvae heterozygous for inversion:		
2R-A	0	20
2R-B	6	0
Chromosome 3:		
No. of test larvae heterozygous for:		
0 inversions	16	50
1 inversion	0	0
2 inversions	11	0
3 inversions	7	0
4 inversions	11	0
5 inversions	18	0
6 inversions	13	0

* See figure 1.

distally. Testes very pale yellow with three outer and two inner coils. Spermatheca heavily chitinized; elongate, almost twice as long as its greatest width; obtuse at apex. Ventral receptacle folded, resembling a skein of wool.

Other characteristics, relationship and distribution

Eggs: Two filaments, ½ the length of the egg, with very broad tips.

Larvae: The larvae do not skip.

Puparia: Yellowish brown; horn very small with about 10 branches.

Chromosomes: Two pairs of V-shaped chromosomes and one pair of rod-shaped chromosomes.

Distribution: Brazil: State of São Paulo: Vila Atlântica (type material, July 1951); Mogi das Cruzes; Horto Florestal, Serra Cantareira, near the city of São Paulo.

Relationship: Belongs to the *willistoni* group of the subgenus *Sophophora*. This species is very closely related to *D. bocainensis* and *D. parabocainensis* but is easily separable morphologically from them in males by the black pleurae.

POPULATION SAMPLES FROM RIO GRANDE DO SUL, BRAZIL

Flies conforming morphologically to the description of *D. bocainensis* Pavan and da Cunha were collected at Feliz and Ponta Grossa in the state of Rio Grande do Sul during July and October, 1951. From each of the 126 wild females which produced offspring in the laboratory, one or more salivary gland chromosome smears of F_1 larvae were prepared. From each of the F_1's, one of the larvae was designated as a "test larva"; this was

normally taken as the first female larva examined, as the use of a female permits the observation of two entire chromosomes of each pair transmitted by the wild-caught female. If no female larva was present among those examined, the first male larva was used; this is less desirable, as only a single X chromosome may be observed.

Inspection of the data from the test larvae reveals a sharp dichotomy (Groups 1 and 2, table 1). In the first place, the female test larvae showed either one of two alternate cytological appearances at the tip of the left arm of the X chromosome (A, B versus C, D, fig. 1). Inspection of the banding differences between these two alternate tips reveals them to be due to the homozygous presence (or absence) of a simple inversion of the segment 2–7, figure 1. Let us arbitrarily designate these two homozygous appearances as AA and aa. In no instance was the expected simple inversion loop of the heterozygote Aa found. This is a highly significant deviation from the Hardy-Weinberg equilibrium, as among the female test larvae, 53 AA, 36 aa and 0 Aa individuals were observed (table 1). The significance of the dichotomy in the data for the X chromosome is further strengthened by the fact that as well as the 126 test larvae reported in table 1, a total of 95 additional larvae were examined; thus a considerable number of the wild flies were tested by more than a single F_1 smear. In no case, in any given F_1, was both types of X chromosome found; the X chromosomes of all the larvae examined from any individual wild female were either all AA or aa (A or a in males) in pattern at the tip of XL.

The separateness of the two groups in table 1 becomes even more clear when the evidence from the other chromosome variability is considered. For instance, 15 of the female test larvae were heterozygous for a short inversion, designated XR-B, near the base of the right arm of the X chromosome. All of these individuals had an AA, or *"bocainensis"* X chromosome. In chromosome 2, 20 test larvae were heterozygous for a short inversion near the tip of the right arm (inversion 2R-A, table 1); all of these had the aa or *"parabocainensis"* X chromosome. Most striking of all, however, is the situation in chromosome 3; those F_1's having *"bocainensis"* X chromosomes show an extraordinary amount of diversity for gene order in chromosome 3. About 80% of these test larvae (60 out of 76) were complex inversion heterozygotes in which the two homologues differed by from two to six inversions. The 50 F_1's which showed the *"parabocainensis"* X chromosome, however, were exclusively homozygous for gene arrangement (table 1).

These data are summarized graphically in the two upper histograms in figure 2; detailed data on the inversions found in *D. bocainensis* are given in table 2. In view of the absolutely discontinuous distribution of the chromosomal variability in the two groups, there is no question that, although they are sympatric and are morphologically very similar, these two groups come from isolated Mendelian populations which do not interbreed. As will be shown later when the data from laboratory crosses between members of these two populations are presented, the above analysis reveals only to a small degree the actual amount of diversity of gene order between the two. Thus, it will be shown that F_1 hybrids between these two entities are heterozygous for from 13–26 inversions, most of which were not detected prior to the species crosses. This diversity is such that even a small amount of introgression between the two entities could be immediately recognized in examinations of test larvae. Although further evidence on this point will be adduced later, the two entities will be hereafter referred to as species.

Population Samples of *D. bocainensis* from São Paulo

Samples of flies were obtained from 1) Vila Atlântica, 2) Serra Cantareira, on

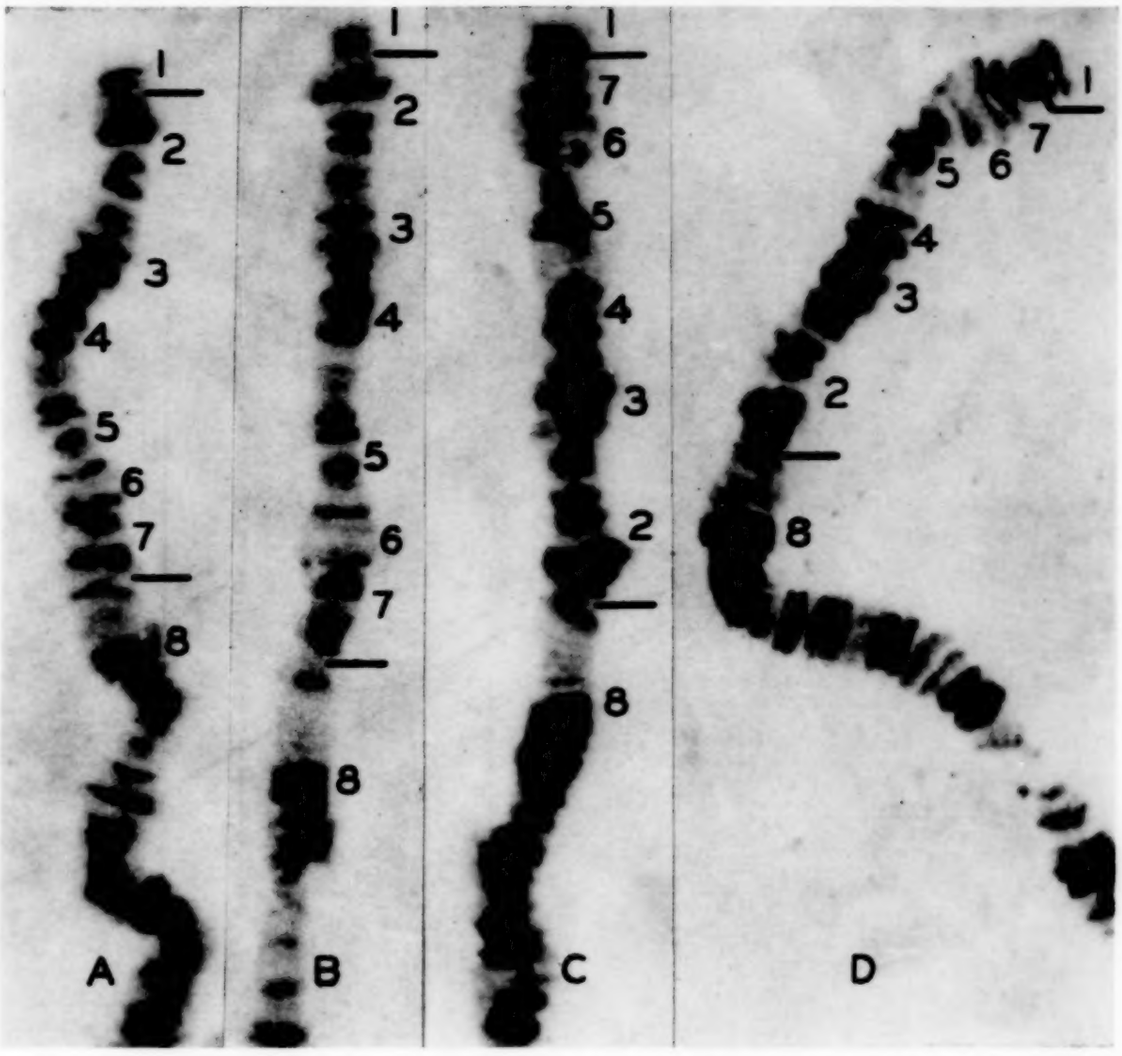

Fig. 1. The tip of XL in *D. bocainensis* (A, B) and *D. parabocainensis* (C, D). The species differ by a subterminal inversion in this region, the breakpoints of which are indicated by the heavy lines. Homologous regions are numbered 1–8.

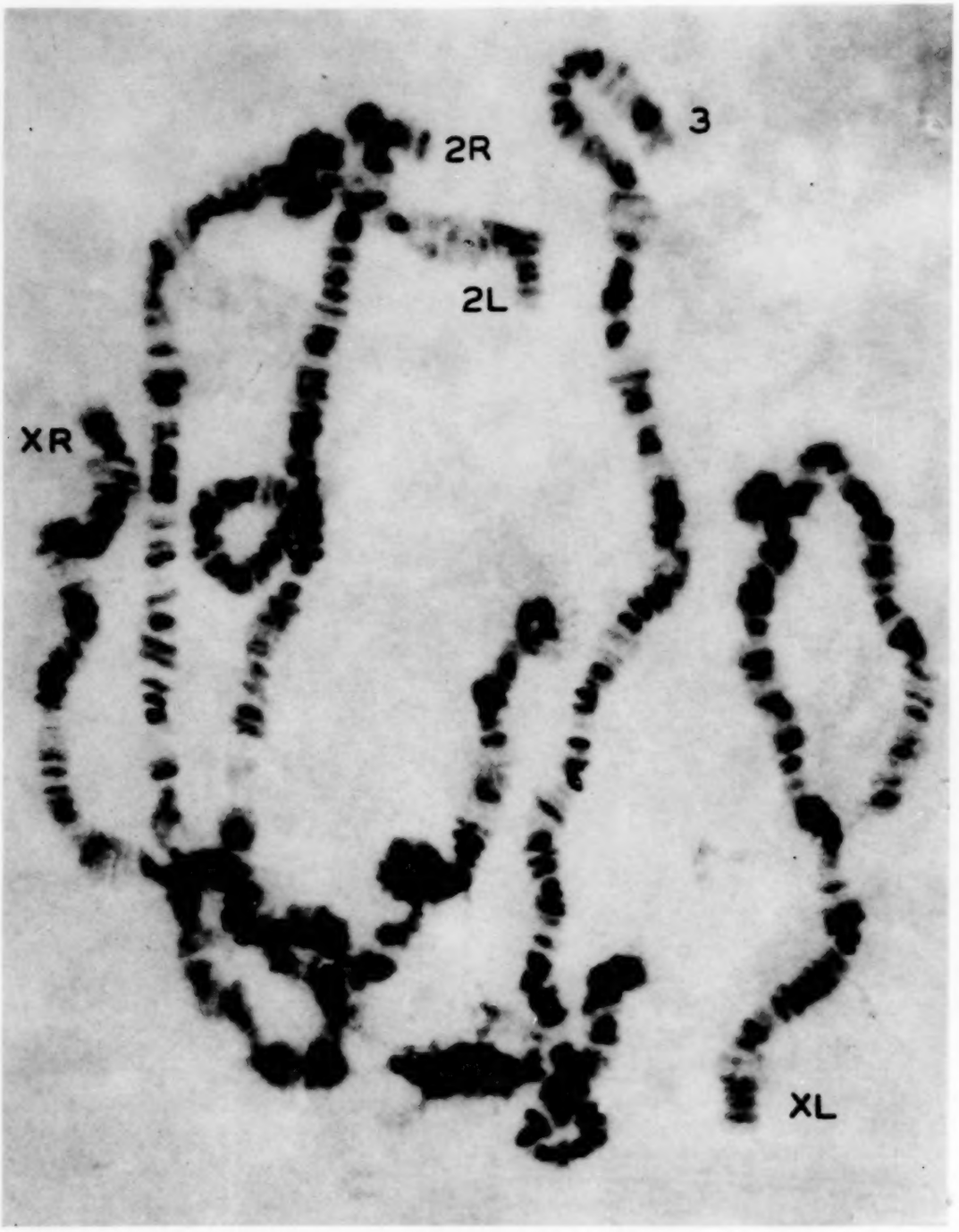

Fig. 4. Entire polytene chromosome complement in an individual of *D. parabocainensis* sp. nov. which is homozygous for gene arrangement. The distal end of each chromosome arm (XL, XR, 2L, 2R, 3) is labelled. XL has broken off from its base (center) and lies to the right of the picture.

the grounds of the Horto Florestal near the city of São Paulo and 3) Mogí das Cruzes. The chromosome variability in the test larvae from flies cytologically determined as *D. bocainensis* is recorded in the three right-hand columns of table 2, where they may be compared with the data from Rio Grande do Sul. A graphic summary of the data for the state of São Paulo is given in the lower histogram in figure 2.

Two inversions not found in the Rio Grande do Sul populations are present in São Paulo; these are the rare inversion XR-A and inversion 2L-A. In general, the frequencies of inversions in chromosomes X and 2 are greater in the São Paulo populations than in Rio Grande do Sul, although the situation with respect to inversion 2R-B is not clear, because of the very small sample obtained at Mogí das Cruzes. In all the populations studied, inversion variability in chromosome 3 is very great and the extent of each individual inversion has not been mapped for this study. There appears, however,

TABLE 2. *Chromosomal variability in* D. bocainensis *Pavan and da Cunha in Brazil, 1951*

	Locality					
	Rio Grande do Sul			*São Paulo*		
	Feliz		Ponta Grossa Oct.	Vila Atlântica July-Sept.	Serra Cantareira Aug.-Sept.	Mogí das Cruzes Nov.
	July	Oct.				
No. of wild females tested	22	38	16	48	106	7
No. tested by single F_1 female larva	12	31	9	34	71	4
No. tested by single F_1 male larva	10	7	7	14	35	3
Chromosome X:						
% of female test larva heterozygous for:						
inversion XL-B	0.0	0.0	22.2	20.6	28.2	50.0
inversion XR-A	0.0	0.0	0.0	2.9	1.4	0.0
inversion XR-B	8.3	35.5	33.3	32.4	45.1	75.0
Chromosome 2:						
% of test larvae heterozygous for:						
inversion 2R-B	9.1	10.5	6.3	2.1	0.0	28.7
inversion 2L-A	0.0	0.0	0.0	20.8	45.7	0.0
inversion 2L-B	0.0	0.0	6.3	33.3	35.2	0.0
inversion 2L-C	0.0	2.6	0.0	33.3	40.0	0.0
Chromosome 3:						
% of test larvae heterozygous for:						
0 inversions	27.3	18.4	25.0	10.4	11.4	0.0
1 inversion	0.0	0.0	0.0	20.8	9.5	14.2
2 inversions	22.7	5.3	18.7	10.4	19.0	14.2
3 inversions	4.5	15.8	0.0	25.0	18.1	0.0
4 inversions	18.2	15.8	6.3	16.7	24.8	28.7
5 inversions	18.2	26.3	25.0	12.5	9.5	14.2
6 inversions	9.1	18.4	25.0	4.2	6.7	28.7
9 inversions	0.0	0.0	0.0	0.0	1.0	0.0

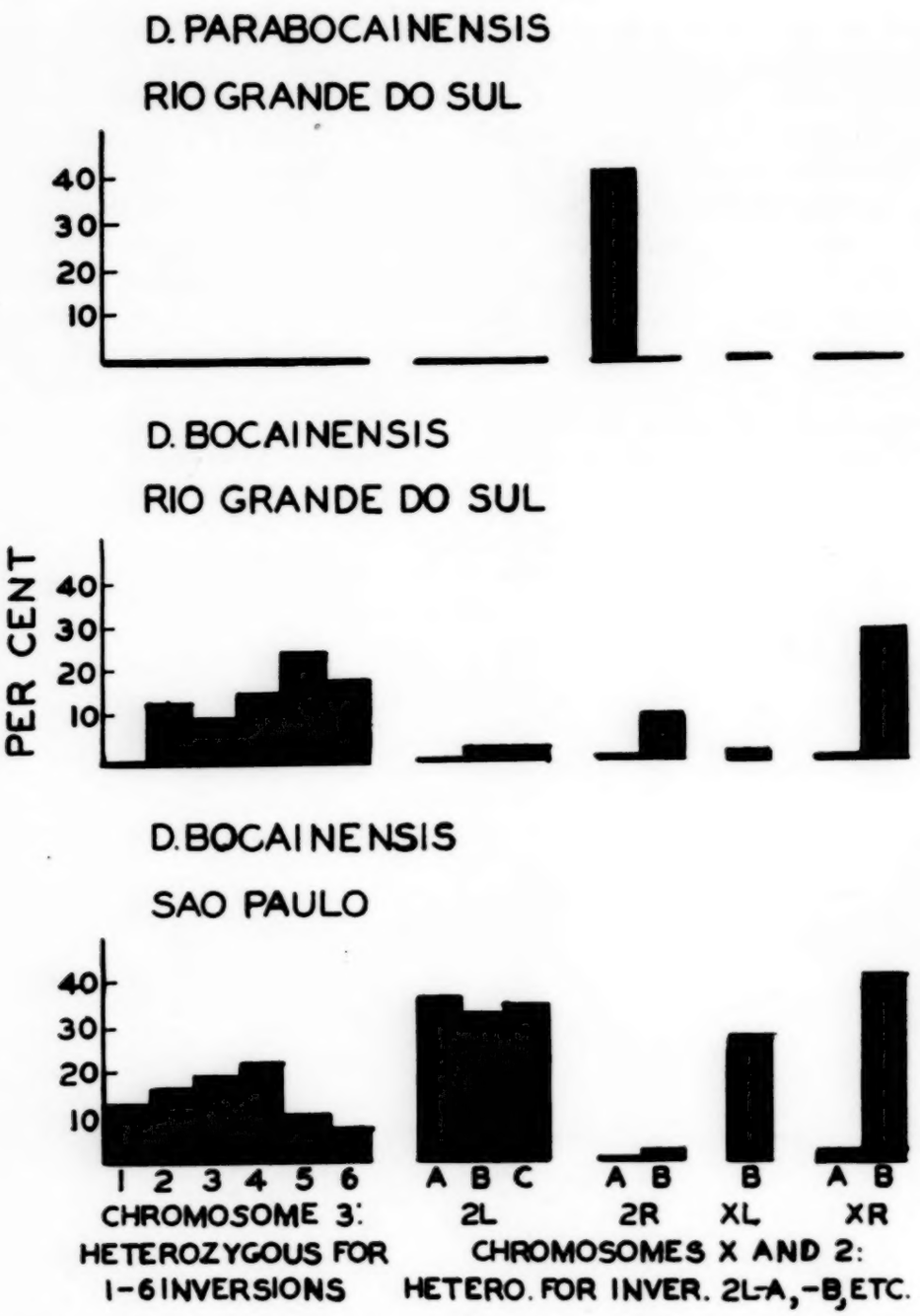

FIG. 2. Chromosome variability within sympatric populations of *D. parabocainensis* (upper histogram) and *D. bocainensis* (middle histogram). Although the differences in variability pattern are apparent, this diagram does not attempt to show all of the differences in chromosomal arrangement between the species. For comparison, São Paulo populations of *D. bocainensis* are summarized (lower histogram). Detailed data are given in tables 1 and 2 and in the text.

to be a maximum of about 10 inversions. The variability in this chromosome has been expressed in terms of the number of inversion differences between the two homologues observed in the test larvae (table 2). This method, however, expresses somewhat less than the total variability encountered, because it is clear that there is a considerable variety of configurations within, for instance, the 1-inversion and 5-inversion difference classes. Despite the inadequacies of the data for chromosome 3, certain differences between the Rio Grande do Sul populations and those

from São Paulo are apparent (table 2; fig. 2). For example, no instance of a single-inversion difference was encountered among the Rio Grande do Sul flies, whereas in the São Paulo populations this class runs as high as 20%. Correlated with this difference appears to be the fact that in São Paulo, a relatively smaller percentage of complex figures (differing by 4–6 inversions) are found, although the individually most complex configuration, a 9-inversion difference, came from the Serra Cantareira.

Population Samples of *D. bocainoides* from São Paulo

At the three locations discussed above, no specimens of *D. parabocainensis* were obtained. Among the *bocainensis*-like flies that were analyzed, however, 35 of the F_1 progenies (23 from Vila Atlântica; 12 from Cantareira) showed banding patterns in the chromosomes which differed markedly from both *D. bocainensis* and *D. parabocainensis*. Close inspection revealed that the general similarities make it possible to homologize the chromosome arms with those of the sibling species, but in most cases direct band homologies could not be drawn.

Separation and examination of the progenies of these females revealed that the emerging flies showed, especially in males, clear morphological differences from the sibling species, the degree of divergence in the chromosomes paralleling, more or less, the morphological divergence. This species is described in this paper as *Drosophila bocainoides* sp. nov. Twelve of the test larvae were heterozygous for a short inversion slightly distal to the center of 2R and three were heterozygous for a short inversion close to the tip of XR. This latter inversion was found only in flies collected at Vila Atlântica. Males of *D. bocainoides* were identified in collections from Mogi, but no females were present among the small sample available from this location.

Population Samples of *D. parabocainensis*

Other than the specimens obtained from Ponta Grossa and Feliz, Rio Grande do Sul, only 9 wild females of this species have been analyzed, 7 from Pirassununga, São Paulo and 2 from Montes Claros, Minas Gerais. In Rio Grande do Sul, the species is homozygous for gene arrangement, except for a short inversion near the tip of 2R (2R-A, table 1) which is present in about 40% of the test larvae. This inversion is also present at Pirassununga and Montes Claros. At these two latter locations, despite the small number of flies analyzed, three additional inversions, all in chromosome 2, have been found. The two inversions found in the left arm (Pirassununga only) resemble 2L-B and 2L-C of *D. bocainensis* but their identity with these inversions has not been definitely established. In the right arm, a large basal inversion has been found in specimens from both Montes Claros and Pirassununga. These data make it probable that when satisfactory samples of the São Paulo populations of *D. parabocainensis* are eventually obtained, the apparent tendency toward inversion homozygosity, relative to *D. bocainensis*, will be shown to be spurious.

Although among the recently-collected *D. parabocainensis* all have been homozygous for gene order in both chromosomes X and 3, examination of an old laboratory stock of this species from Pirassununga revealed that it was carrying a chromosome 3 which differed from its homolog by five inversions. The possibility of contamination of this stock with *D. bocainensis* cannot be excluded.

Hybridization Experiments Between *D. parabocainensis* and *D. bocainensis*

1. P_1 *hybridizations*

Reciprocal crosses between *D. parabocainensis* and *D. bocainensis* were made, using F_1 flies from wild females (table 3).

TABLE 3. *Results of P_1 reciprocal crosses between* D. parabocainensis *and* D. bocainensis. The fractions given indicate the number of tubes out of the total in each category which produced abundant offspring. Each tube contained 5 males and 5 females.

A. *parabocainensis* females × *bocainensis* males

		Parabocainensis females	
		Feliz	Pirassununga
Bocainensis males	Feliz	5/5	0/1
	Vila Atlântica	5/5	1/1

B. *bocainensis* females × *parabocainensis* males

		Bocainensis females	
		Feliz	Vila Atlântica
Parabocainensis males	Feliz	8/8	6/6
	Pirassununga	2/3	2/2

For each test, 5 virgin females and 5 males of approximately the same age were placed together in a large shell vial and each tube was examined daily for the appearance of eggs and larvae. A total of 18 strains were employed in the crosses: 7*parabocainensis* from Feliz, 1 *parabocainensis* from Pirassununga, 5 *bocainensis* from Feliz, and 5 *bocainensis* from Vila Atlântica. A series of 17 control (intraspecific) crosses were made at the same time and were prepared and followed in the same way.

Of 31 interspecific crosses made, 29 produced abundant larvae at the same time and in the same quantity as the controls (table 3). The fact that two of the crosses failed is not considered significant in view of the fact that of the 17 intraspecific control crosses, three also failed. All of the latter failures were intrastrain matings of *D. bocainensis*. No difference was noted in the success of sympatric and allopatric crosses. The interspecific crosses did not differ from the controls in abundance of larvae produced, time of pupation and emergence of adult flies. No dying larvae or pupae were observed and the F_1 hybrid flies were fully as vigorous as the controls, if not more so.

Study of the sex ratio in the F_1 generation from the crosses described above was carried out on 7 of the crosses, 2 Feliz × Feliz, 3 Feliz × Vila Atlântica and 2 Vila Atlântica × Pirassununga. The data were obtained by isolating the five individual females used in each cross after they had already produced larvae in several successive tubes. Each female was placed without males in a separate tube and changed regularly to a fresh one until her supply of sperm was exhausted. The F_1 hybrid individuals were thus raised under conditions where no crowding occurred and all individuals emerging were counted. The results of these counts are given in table 4. It will be noted that whereas the sex ratio among the allopatric crosses did not significantly deviate from a 1 : 1 ratio, there is a highly significant shortage of

TABLE 4. *Sex ratios in the offspring of reciprocal crosses between* D. parabocainensis *and* D. bocainensis

	Sex ratio in F_1 hybrids					
	Sympatric (Feliz × Feliz)			Allopatric (Feliz × Vila Atlântica) (Vila Atlântica × Pirassununga)		
P_1 cross *female* × *male*	No. females	No. males	Sex ratio	No. females	No. males	Sex ratio
para. × *boca.*	125	93	74.4	220	199	90.5
boca. × *para.*	295	166	56.3	249	269	108.0
Total	420	259	61.7	469	468	99.8

TABLE 5. *Fertility of interspecific hybrids between* D. parabocainensis *and* D. bocainensis. The fractions given indicate the numbers of tubes out of the total in each category which produced F_2 offspring. ++ indicates abundant offspring produced; + indicates few offspring.

A. tests of F_1 hybrids from *D. parabocainensis* female × *D. bocainensis* male

Test No.	female × male	Feliz × Feliz sympatric	allopatric
1	hybrid × hybird (mass)	++ 4/4	++ 5/5
2	hybrid × hybrid (tubes)	++ 2/2	++ 2/2
3	hybrid × *para.*	++ 2/2	++ 2/2
4	hybrid × *boca.*	++ 1/2	++ 2/2
5	*para.* × hybrid	++ 1/2	++ 2/2
6	*boca.* × hybrid	++ 2/2	++ 2/2

B. Tests of F_1 hybrids from *D. bocainensis* female × *D. parabocainensis* male

	female × male	Feliz × Feliz sympatric	allopatric
7	hybrid × hybrid (mass)	++ 7/7*	+ 3/9†
8	hybrid × hybrid (tubes)	− 0/2	− 0/2
9	hybrid × *para.*	++ 2/2	++ 2/2
10	hybrid *boca.*	+ 1/2	+ 2/2
11	*para.* × hybrid	− 0/2	− 0/2
12	*boca.* × hybrid	− 0/2	− 0/2

* Delay 1–5 weeks; 4 of the 7 with abundant offspring.
† Delay 2–5 weeks; none of the 3 with abundant offspring.

males from the sympatric cross, regardless of the way in which the original cross is made. This effect is noticeable in the progenies of each of the 9 individual females which produced offspring; in no individual progeny does the number of males equal or exceed that of the females.

2. *Fertility of F_1 hybrids*

The fertility of the F_1 hybrid individuals was tested by mass matings of F_1 individuals from 25 of the successful crosses. Four of the original crosses (reciprocals; sympatric and reciprocals; allopatric) were selected for more intensive tests by F_1 matings and backcrosses. These data are summarized in table 5.

Of 9 original crosses in which *D. parabocainensis* was the female parent, all produced vigorous F_2 generations in mass cultures containing approximately 25 individuals of each sex, as shown in test No. 1, hybrid × hybrid (mass), table 5. The sympatric crosses were equally as successful as the allopatric ones (Feliz × Vila Atlântica and Pirassununga × Vila Atlântica). The abundance of offspring equalled that of the controls and F_2 larvae were produced without delay (6–8 days after emergence). Two of the original crosses (Feliz × Feliz No. 2 and Feliz × Vila Atlântica No. 2) were selected for more intensive study. Tubes containing 5 females and 5 males each were prepared of F_1 hybrids (test No. 2, table 5) and backcrosses (tests No. 3–6, table 5). As might be expected from the results obtained in mass cultures, all combinations showed a high degree of fertility. The fact that one of the tubes failed in each of tests 4 and 5 is not considered significant in view of the fact that abundant offspring were produced in each case in the duplicate tube.

Tests of F_1 hybrid individuals from the 16 crosses which were the reciprocal of the above, that is, in which *D. bocainensis* was the female parent, were tested in a similar manner in mass cultures (test No. 7, table 5) and in tubes (tests 8–12, table 5). It was soon apparent that, compared to the hybrids from the reciprocal cross and the controls, some degree of sterility existed. For this reason all of the tests

7–12 were followed intensively for a period of two months, during which time the flies were periodically transferred to fresh food and any dead flies in the crosses being followed in tubes were replaced with fresh ones.

Using this procedure, all of the sympatric crosses of this type were observed to produce offspring, although there was a delay of from 1–5 weeks beyond the control time for the production of offspring. Four of these crosses eventually produced abundant offspring, whereas the other three produced only a small F_2. F_1 hybrids from the original allopatric crosses in mass culture were noticeably less successful. Of 9 such crosses followed, only 3 produced F_2 larvae. Although egg production by the females was enormous over the two-month period, very few offspring were obtained; in all cultures the appearance of larvae was delayed from 2–5 weeks beyond the normal time. Of 5 crosses of Feliz by Vila Atlântica, only two produced offspring; one of these resulted in the hatch of only 1 female, the other produced 8 females and 1 male. Two crosses of Vila Atlântica × Pirassununga were negative and of two crosses of Feliz × Pirassununga, one was sterile and the other produced 16 females and 13 males.

Two of the reciprocal crosses in which *D. bocainensis* was the female parent were followed in detail, as explained in a previous section. These were Feliz × Feliz No. 3 and Vila Atlântica × Feliz No. 5. The results are given in tests 8–12, table 5. It will be noted that all tests involving F_1 hybrid males failed (tests 8, 11, 12, table 5) despite the fact that they were carried for two months. F_1 hybrid females of this type proved to be highly fertile when backcrossed to *D. parabocainensis* males (test 9). The backcrosses to *D. bocainensis* males were somewhat less successful, but 3 out of 4 such tests produced moderate numbers of offspring.

The discrepancy between the results of mass cultures and tube experiments and backcrosses (tests 7–12, table 5) may be best explained as indicating that the F_1 hybrid males from the cross *D. bocainensis* female × *D. parabocainensis* male are only occasionally fertile and become so only after considerable delay. Thus, the success of the mass cultures may have been due to the fertility of a very few males or conceivably a single male. All hybrid females, in fact most of the species females as well, produced enormous quantities of eggs when in tubes with sterile males; in a number of cases, the hybrid females were still active and vigorous and were producing large quantities of eggs at the time they were discarded, two months from the time of emergence. A number of putative sterile hybrid males were dissected; in all cases the testis resembled that of the species controls and of fertile hybrid males from the reciprocal cross; motile spermatozoa were observed in the ejaculatory duct. These males are furthermore able to inseminate females; dissections of their mates revealed that the seminal receptacles were filled with actively motile sperm. Examination of eggs laid by these females, however, showed them to be uncleaved.

3. *Crosses involving* D. bocainoides

As has been stated previously, this species is, on morphological and cytological grounds, somewhat removed phylogenetically from the sibling species discussed above. *D. bocainoides,* moreover, is difficult to rear in the laboratory. Six hybridization attempts, two with *D. bocainensis* and four with *D. parabocainensis* were made, using 5 flies of each sex as in the experiments described above. No offspring were obtained and further attempts were abandoned.

Salivary Gland Chromosome Studies of Interspecific Hybrids, F_2's and Backcrosses

Studies on F_1 larvae from wild females, described in an earlier section, have shown

TABLE 6. *Chromosomal differences between* D. bocainensis *and* D. parabocainensis *as revealed in species crosses and in studies of intraspecific variability*

Chromosome arm	Number of inversions observed		
	Minimum		Maximum
XL	4		5
XR	5		7
2L	0		5
2R	0		2
3	4		7
Total	13	range	26

that there is considerable divergence of gene order within one of the species (*D. bocainensis*) and at least one consistent difference (an inversion near the tip of XL) between the species. This method of analysis, involving the recognition of inversion heterozygotes and chromosome ends, reveals only a small measure of the differences of gene order between these species. This fact is apparent from the study of the chromosomes of F_1 hybrids. The salivary gland chromosomes of a total of 47 F_1 hybrid larvae were examined and proved to be heterozygous for from 13 to 26 inversions, depending on the strain of *bocainensis* used in the cross (table 6). In the crosses from Feliz, strains of *D. bocainensis* homozygous for gene arrangement in the X chromosome were used. As all strains of *D. parabocainensis* are entirely homozygous for gene arrangement in this chromosome, such crosses should reveal the basic differences in gene order between the species. In addition to the nearly terminal inversion in XL (the configuration Aa, predicted from the two homozygous appearances in the two species), all larvae were heterozygous for 8 additional inversions in the X chromosome. Thus, in the interspecific hybrids, XL shows four inversions, a simple subterminal one (Aa), an overlapping inversion figure centrally and another single inversion proximally. In XR there are 5 inversions, bearing a complex relationship to one another.

In most of the crosses examined, the 3rd chromosome forms excessively complicated figures which are difficult to analyze. In one case the difference amounted to 8 inversions. A great variety of these complex figures was found in the larvae because of the widely diverging gene orders found within *D. bocainensis*. Chromosome 2 represents an interesting case in that unlike the X (and apparently the 3rd) chromosome, the two species share a common gene arrangement. Thus, in the right arm, the interspecific hybrids show either two inversions (2R-A and 2R-B), 2R-A alone, 2R-B alone, or no inversions. The latter condition was recorded in a Feliz by Feliz cross. In the left arm of chromosome 2, the most common condition in the F_1 hybrids is a very complex configuration which has not been satisfactorily analyzed, although it appears to be due to a 5-inversion difference. In two instances, however, the difference between the two was simpler; in crosses between Vila Atlântica and Pirassununga some crosses showed only a two-inversion difference and in one instance the gene arrangement in the two species proved to be the same, that is, no inversions were found. These observations show that the two species share a common gene arrangement in both 2L and 2R. A more detailed analysis is necessary before the derived arrangements found within the species may be clarified relative to a "standard" arrangement.

An examination of the salivary gland chromosomes of 28 larvae of the F_2 generation and backcrosses was made. Although this examination was cursory, the following facts are clear. Segregation in the F_2 is random and involves principally whole chromosomes in both males and females. A number of recombinations between arms were also detected. Thus, in several cases it was observed in chromosomes X and 2 that a given hybrid female transmitted a right arm derived from one species along with a left arm derived from the other.

Geographical Distribution and Ecology

Most species of Drosophila which have been subjected to detailed microevolutionary studies are relatively abundant in banana-bait collections, at least seasonally or locally. Wild-caught adults of these species, unless in some state of reproductive arrest, usually breed readily when brought to the laboratory and may be maintained on standard laboratory media with little difficulty. The members of the *bocainensis* subgroup unfortunately do not display a full measure of these favorable attributes. Like perhaps the majority of species of the genus, they show ecological specialization; concomitant technical difficulties occur if they are handled by routine laboratory methods.

In the first place, flies of this subgroup are exceedingly rare in banana trap collections. This might be taken to mean that populations are generally small were it not for the fact that the evidence indicates rather strongly that under most circumstances fermenting banana does not attract the flies well. The most striking evidence for this fact comes from the collecting activities of Pavan (1952) at Vila Atlântica during September, 1951. Approximately one week of collecting from banana baits resulted in a catch of only 18 *bocainensis* subgroup out of 6920 flies captured, or 0.3%. Fermenting orange, hitherto suspected of being a good attractant for these species, yielded only 0.4% out of 1325 flies caught. That neither of these baits was adequately sampling the population in the area, however, is shown by the fact that of 1472 Drosophila swept from rotting *Myrciaria* [1] fruits in one restricted spot on the forest floor near the collecting area, 462, or 31.4%, were *bocainensis* subgroup.

From these data, it is clear that *bocainensis* subgroup (specifically, about 75% *D. bocainensis* and 25% *D. bocainoides*) were in this case selectively attracted to the wild fruits, and if the collecting at Vila Atlântica at this time had been made exclusively from banana baits, an erroneous impression of the size of the *bocainensis* populations would have been obtained. As most of the routine sampling of South American populations of Drosophila has been done with banana bait (e.g., Dobshansky and Pavan, 1950; Pavan, 1952) it is probable that published figures on the incidence of *D. bocainensis* do not accurately reflect the state of its natural populations.

The ecological relationships of the flies of the *bocainensis* subgroup to fruits of trees of the family Myrtaceae, especially of the genus *Myrciaria,* are much in need of investigation. Good collections of these species have been made from wild fruits of members of this genus on a number of occasions. Preliminary tests of commercial relatives of these wild species of trees indicate that certain of their fruits are promising as specific attractants. The fruits tested were: jaboticaba, *Myrciaria* (*Eugenia*) *jaboticaba* or *M. cauliflora* and uvaia, *M. uvalha* or *M. pyriformis.*

Pavan (1952) has recorded small numbers of flies of this subgroup from various of the northern states of Brazil (Amazonas, Maranhão, Goyaz, Acre) as well as from the south (São Paulo, Paraná). Table 7 gives the geographical origin and relative frequency in collections made with banana or orange bait of the three species recognized in the present paper. All determinations, except as noted, are based on cytological examination.

Despite the doubtful attractant qualities of banana and orange bait, as discussed above, several facts are apparent. In the first place, the data from Feliz, Rio Grande do Sul, suggest the existence of a reciprocal seasonal cycle of abundance of the sibling species *D. bocainensis* and *D. parabocainensis,* with the latter the more frequent species in winter (July). As the data stand, the change from July to October in the frequency of these two

[1] *Myrciaria delicatula* (B. C.) Berg, family Myrtaceae. Kindly determined by Dr. M. G. Ferri.

TABLE 7. *Geographical distribution and relative frequency of* Drosophila bocainensis, D. parabocainensis *and* D. bocainoides *in Brazil.* All records verified cytologically, except as indicated.

Locality	Total	Per cent		
		bocainensis	*parabocainensis*	*bocainoides*
Rio Grande do Sul, 1951	129	61.2	38.8	0.0
Feliz, July	51	43.1	56.9	0.0
Feliz, October	54	75.9	24.1	0.0
Ponta Grossa, October	24	66.7	33.3	0.0
São Paulo, 1951–52	206	83.0	0.0*	17.0
Vila Atlântica	72	68.1	0.0	31.9
Cantareira	120	90.0	0.0*	10.0
Mogí das Cruzes	7†	95.0	0.0	5.0
Pirassununga	7	100.0	0.0	0.0
Minas Gerais, 1953				
Montes Claros	3	33.3	66.7	0.0

* Mr. F. M. Salzano reports the presence of this species in a collection from Cantareira in 1952.
† All 7 flies tested cytologically were *D. bocainensis.* The percentages are based on the relative number of *bocanioides* males in the collection.

species is highly significant statistically but the possible effect of many intangible and uncontrolled factors make statistical comparisons of relative frequency open to question. For example, both orange and banana were used in the October collections and the collection period extended over a number of days. A single collection, using banana only was made in July. The species are not spacially separated in the forest; both may be captured over the same bait pile with a single sweep of the net.

The high frequency of *D. parabocainensis* at the cooler, drier season in Rio Grande do Sul correlates directly with its occurrence, so far to the exclusion of *D. bocainensis,* at Pirassununga, São Paulo. This station is located on the dry interior plateau. Conversely, *D. bocainensis,* with large populations at Vila Atlântica and in the Cantareira is apparently a fly of the coastal rain and cloud forest. *D. bocainoides* is definitely less frequent at the higher altitudes near the city of São Paulo than it is on the coast, suggesting that its distribution may be more characteristic of areas of high temperature, rainfall and humidity than the other two species.

In most cases, the number of specimens originally captured was much greater than the number which could be successfully analyzed cytologically. This is due primarily to the fact that many wild females of this subgroup lay only a few or no eggs after being brought to the laboratory. Wild-caught flies of both sexes feed well in the laboratory and live for long periods if changed periodically to fresh food. Disinclination to oviposit in the laboratory appears to be about equally pronounced in the three species. As far as general laboratory culture is concerned, all three species may be raised on banana food; *D. parabocainensis* is the most vigorous and is the easiest to raise. *D. bocainoides* requires the most attention; careful aging and repeated changes of the flies are necessary to assure well-populated culture bottles. Hybrid stocks are highly vigorous and have been maintained without difficulty for several years (Carson, 1953).

CYTOLOGICAL SYNOPSIS

The X chromosome

General. The left and right arms of the X chromosome are joined by a small heterochromatic mass at the chromocenter region. As in the *D. willistoni* subgroup and in *D. nebulosa,* XL is shorter than XR (fig. 3). The banding pattern at the tip of XR appears to be identical

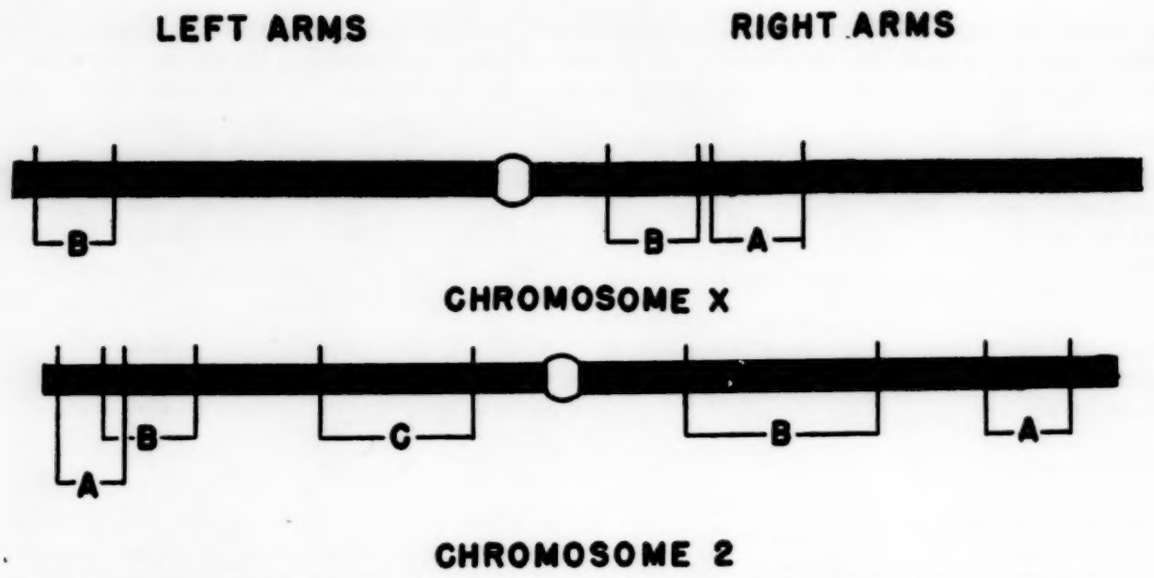

FIG. 3. Diagrams of the left and right arms of chromosomes X and 2 of *D. bocainensis* and *D. parabocainensis*. The light areas in the middle represent the position of the heterochromatin and the centromeres; the bracketed lettered sections refer to inversions XL–B, XR–B, etc. All of the inversions shown, with the exception of 2R–A, are characteristic of *D. bocainensis* only.

in all three species of this subgroup; this pattern, furthermore, appears to be very similar to the regions of like position in *D. willistoni* (region 36, Dobzhansky, 1950) and *D. nebulosa* (region 35, Pavan, 1946). The banding pattern at the tip of the left arm serves as the easiest cytological means to distinguish *D. bocainensis* from its sibling species, *D. parabocainensis* (fig. 1; see also XL, fig. 4). The difference is due to an interspecific inversion, designated as XL-A.

D. bocainensis. Left arm pattern commonly as in figures 1A and 1B. Only a single inversion is known within the species (XL-B, fig. 3). The distal break of this inversion is just proximal to the three large bands designated "2" in figures 1A and 1B. Although the appropriate mapping has not been done, it is probable that this inversion overlaps XL-A at its proximal end. Two inversions are known in the right arm; XR-B is a very short inversion close to the base and XR-A is immediately distal to it and is about the same length (fig. 3).

D. parabocainensis. The tip of the left arm banding is exclusively as in figures 1C and 1D; no inversions in either arm are known within the species. In crosses to *D. bocainensis* which lacks XL-B, XR-A and XR-B, the hybrid shows regularly four inversions in the left arm; a nearly terminal single inversion (XL-A), two inversions which overlap centrally in the arm and a single large inversion near the base. Interspecific hybrids show a complex figure in the right arm which is apparently due to a five-inversion difference.

D. bocainoides. The terminal portion of XL cannot be homologized with either of the siblings; XL is best identified by elimination, as the pattern at the tip of XR makes this arm easily identified. A short inversion (XR-C), with its distal break seven bands from the tip, occurs within the species.

Chromosome 2

General. The left and right arms of the second chromosome are usually joined by a large, conspicuous heterochromatic mass, as in *D. willistoni* and *D. nebulosa.* As in the latter two species, 2L may be recognized by the fact that it is shorter than 2R and contains much interstitial heterochromatin, causing it to be kinky and ordinarily unfavorable cytologically.

D. bocainensis. Three inversions are known in the left arm, 2L-A, 2L-B, 2L-C (fig. 3). These may be most easily recognized by their positions in the arm. In the right arm, this species has a single long inversion (2R-B) located centrally in the arm, somewhat more towards the base than the tip.

D. parabocainensis. The left arm is not completely analyzed (see section on hybrids). Inversions are present in both the left and right arms. They are not given letter designations here, however, because their relationship to *bocainensis* inversions of similar position have not been determined. 2R-A is a very common inversion near the tip of the arm; it is the only inversion known from all populations of this species. It does not occur within *D. bocainensis.*

D. bocainoides. The banding patterns at the tips of the second chromosome arms are not recognizably homologous to the sibling species. 2L is easily recognized, however, by its interstitial heterochromatin. There is a short in-

version (2R-C) near the center of the right arm.

Chromosome 3

This element, which is the rod-shaped chromosome of the metaphase group, is easily recognized in both the *bocainensis* and *willistoni* subgroups and in *D. nebulosa* by the fact that it has very little or no heterochromatin at its base. In salivary gland preparations, it appears as a single chromosome arm which tends to separate from the rest of the chromosome group (fig. 4). As has been shown previously, there is a large number of inversions in this chromosome in *D. bocainensis;* natural populations of *D. parabocainensis* and *D. bocainoides,* however, are apparently homozygous for gene arrangement in this chromosome.

Discussion

The implications and uses of the term "sibling species" have been fully discussed by Mayr, Lindsley and Usinger (1953). The term is commonly used to designate two or more non-interbreeding Mendelian populations or species, the individuals of which are so similar morphologically that often separation of the species by one or more qualitative characters is not possible. The most significant recent development in this field is not the abandonment of morphological criteria alone for the recognition of species, a step which was taken long ago, but the development of highly refined cytogenetic and experimental methods for the recognition of sibling species. In cases where these methods can be readily applied, the list of valid cases continues to lengthen.

The present case of sibling species was uncovered by cytological means. The situation is especially clear because the differences in gene sequence between the species are extensive and well-distributed over the chromosome set. Thus even a small amount of interbreeding in nature could be quickly and easily detected in the chromosomes of larvae from wild females. None was detected and like the majority of other cases of sympatric sibling species known in *Drosophila,* isolation in nature appears to be complete.

The completeness of the isolation between the two species in nature is all the more remarkable because there is little or no easily recognized isolation between them in the laboratory. This is in sharp contrast to the situation in the other case of sibling species in the same group of flies, the *willistoni-paulistorum-tropicalis-equinoxialis* cluster (Burla et al., 1949). In these cases no interspecific offspring are produced either in nature or in the laboratory. In *bocainensis-parabocainensis,* furthermore, the species hybrids are not only produced in abundance but, as is perhaps the most unique feature of the present case, all types of hybrids are fertile, with only one type of male having fertility reduced relative to the controls.

The case in *Drosophila* which most closely parallels that of *bocainensis-parabocainensis* is that of *pseudoobscura-persimilis* (Dobzhansky and Epling, 1944). In this case, the entities concerned are also sympatric; crossing in nature does not occur and reciprocal laboratory crosses between the species result in abundant offspring. The main point of difference is that in *pseudoobscura-persimilis,* the F_1 hybrid males are sterile, no matter which way the original cross is made.

The germ plasms of *D. bocainensis* and *D. parabocainensis,* a pair of species apparently formed by divergent speciation, are thus compatible and are still capable of being easily fused. This has been done experimentally in the laboratory; two of four laboratory strains kept for 30 generations still retained some chromosomes derived from both species (Carson, 1953). Under natural conditions, however, the species have remained separate. It is highly unlikely that the small amount of hybrid sterility found could play more than a minor role, if any, in keeping the two species apart. This sterility is better interpreted as a by-product of the speciation process and it appears more likely that some subtile but efficient ecological separation exists in natural populations. This need not be an actual spacial separation; indeed, both species may be col-

lected over baits with a single sweep of the net and such separation appears to be excluded, at least where artificial baits are used. The potentiality for crossing in nature between these species is certainly very great and might conceivably lead to introgression under conditions of disturbance of the delicate ecological balance between them (cf. Anderson, 1948).

That the number of known cases of sibling species should continue to increase for the genus *Drosophila* is not surprising. As a genus, these flies may be looked upon as small flying insects of conservative form which have become adapted, through extensive speciation, to exist on some microorganism or constellation of microorganisms. In other words, the major factors of organic diversity to which these flies are adapting in their evolution is itself morphologically cryptic but at the same time highly diverse biochemically. Just as complex morphological flower form and complex morphology of pollinating insects are correlated in their evolution, so one might expect "sibling species" among yeasts, for example, to encourage the formation of correlated sibling species among the flies adapted to them.

Summary

1. The *bocainensis* subgroup of the *willistoni* group of Drosophila consists of three species from southern Brazil. Two are described in this paper.

2. *D. bocainensis* and *D. parabocainensis* are sibling species which are indistinguishable morphologically. They occur together in the same collections but chromosome analyses prove that crossing in nature does not occur. The species differ profoundly in gene arrangement (13–26 inversions).

3. Despite the lack of natural hybridization, laboratory crosses between the species are highly successful and resemble intraspecific ones in abundance and vigor of F_1 progeny. All hybrids from both reciprocal crosses appear to be as fertile as the controls except for F_1 males which have *bocainensis* mothers. In these, fertility appears to be strongly reduced.

4. It is suggested that the sibling species are kept apart in nature by unknown subtile differences in ecological factors to which the species are differentially adapted.

5. *D. bocainoides* is morphologically distinguishable from and will not hybridize with the sibling species. Data on chromosome variability within each of the three species is given.

6. *D. parabocainensis* appears to be a fly of the dry, seasonally cool interior plateau of southern Brazil, whereas *D. bocainoides* is characteristic of the superhumid coastal rainforests of São Paulo. *D. bocainensis* appears to thrive in intermediate habitats. All appear to be relatively specialized and to have some particular relationship to fruits of trees of the genus *Myrciaria* (Myrtaceae).

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EVIDENCE OF TOOTH STRUCTURE ON THE RELATIONSHIPS OF THE EARLY GROUPS OF CARNIVORA

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INTRODUCTION

Tooth structure provides much of the evidence upon which the classification of the early Tertiary carnivorous mammals has been based. Such classifications in current use divide the terrestrial Carnivora into two suborders, the primitive, polyphyletic Creodonta, and the progressive and extant Fissipeda. Good examples of such classifications are those of Matthew (1909, 1915) and Simpson (1945).

Whatever the arrangement of the higher categories may be, there is general agreement on the number and composition of the creodont families. These are represented diagrammatically in figure 1 to show their chronological relationships without suggesting phylogeny. The earliest family in appearance in the fossil record is the Arctocyonidae, well represented in the Puercan fauna, which is the oldest known assemblage of Tertiary mammals. Arctocyonids are mostly small forms, with delicate dentitions showing little trend towards the shearing function. In some later genera, such as *Arctocyon*, a relatively large size was achieved, and the dentition was blunt and bear-like. The Arctocyonidae constitute the Procreodi of Matthew and the Arctocyonoidea of Simpson. By Middle Paleocene time another family, the Mesonychidae, had made their appearance. Mesonychids ranged from small to very large in size and had the dentition peculiarly modified. Matthew called this group the Acreodi and Simpson placed it in its own superfamily, the Mesonychoidea. The remaining creodont families all have the dentition developed for shearing by the modification of certain teeth. These are the carnassial or sectorial teeth. The upper carnassial is always, in rank at least, the tooth ahead of the lower tooth against which it shears. For example, the carnassials of fissipeds are the last upper premolar and the first lower molar. If the upper carnassial is not the last tooth, there is usually a change in the direction of the tooth row immediately behind. On the basis of the carnassial teeth the families Oxyaenidae, Hyaenodontidae, and Miacidae can be separated. Matthew grouped the first two families as the Pseudocreodi and designated the Miacidae as the Eucreodi. Simpson substituted the superfamily Oxyaenoidea for the tribe Pseudocreodi and removed the Miacidae to the Fissipeda.

Although phylogenetic inferences have been avoided in the above summary, writers such as Matthew, Simpson, and Gregory and Hellman (1939) have dealt with classification and phylogeny in the same discussion. These authors have regarded the Creodonta as a natural group, with the Arctocyonidae as the ancestral family, from which diverged the other lines, including the ancestors of the Fissipeda (Miacidae). It is the purpose of the present paper to call attention to certain aspects of molar structure that indicate a different relationship of the creodont families. Let us consider the characteristic molar pattern in each of these families.

ARCTOCYONIDAE

Disregarding the Arctocyoninae, the ursoid branch, members of this family were small mammals, with dentitions resembling those of the dilambdodont insectivores. The seemingly primitive tooth structure, together with the early time of appearance of the family, has led students to regard the Arctocyonidae as the stem

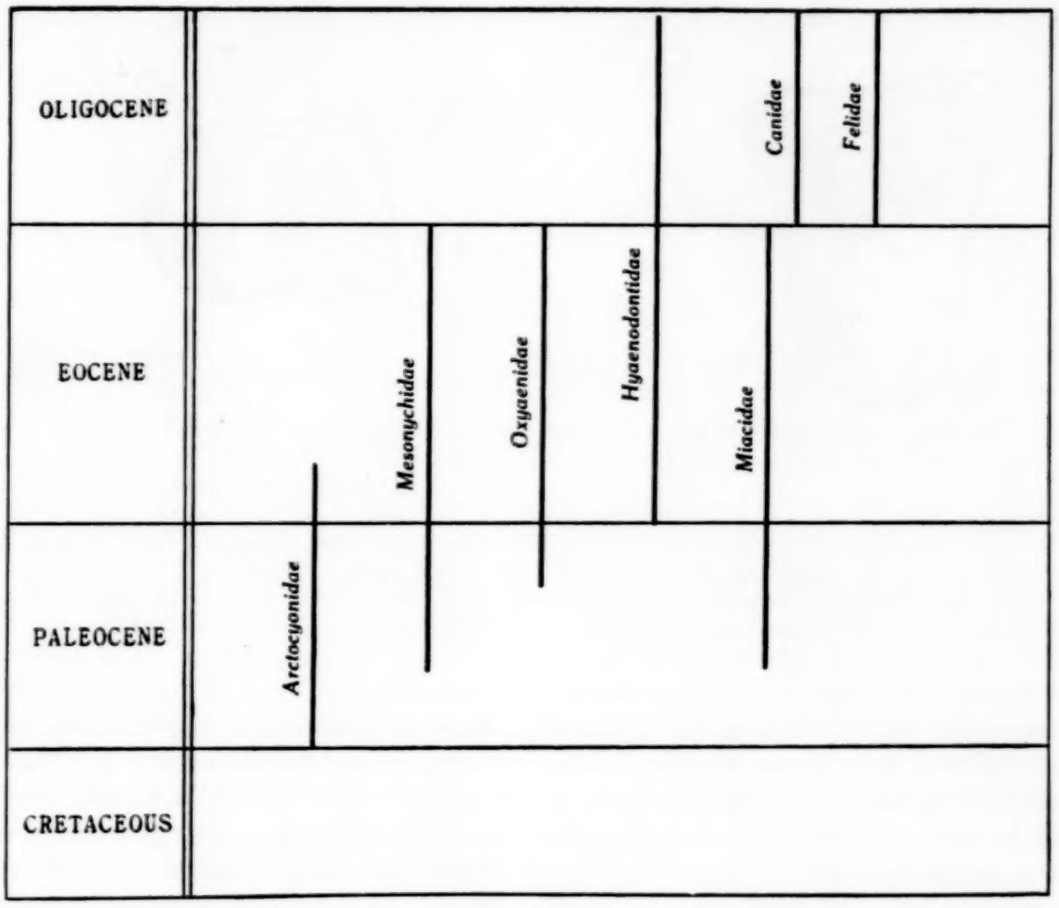

FIG. 1. Time ranges of the early families of Carnivora; duration of epochs not to scale.

group from which the other creodont lines arose.

Let us consider the structure of the molar teeth in the Puercan genera of Arctocyonidae, such as *Oxyclaenus* (fig. 2), *Loxolophus,* and *Protogonodon.* In all of these genera the M^1 and M^2 bear a small but distinct hypocone arising from the posterointernal portion of the cingulum. Even more significant features are to be seen in the lower molars, where the trigonid is smaller and more constricted than the talonid, and the paraconid is partially suppressed. These features, as will be shown, are in direct contrast to the trend in the other creodont groups, where the upper molars develop a hypocone very late or not at all, and where the lower molars have a large trigonid, with prominent paraconid. The last is the most significant feature, for in all creodont groups except the Arctocyonidae the paraconid is the second most important cusp on the trigonid. The metaconid is a reduced cusp, whereas in the arctocyonids it equals the protoconid in size.

These features that distinguish the arctocyonid molars from those of other creodonts are found in greatly exaggerated form in the group of primitive ungulates known as the Condylarthra. The molars of a typical Paleocene condylarth such as *Tetraclaenodon* (fig. 3) are much more robust and rectangular than those of most arctocyonids, but the trends in cusp development are the same. The hypocone, evidently derived from the cingulum as in arctocyonids, is here in process of being taken into the crown pattern to produce a quadritubercular molar such as is basic to all of the ungulate dentitions. Quadrituberculy in the lower molars is being achieved by the suppression of the paraconid and the subequal development of protoconid, metaconid, hypoconid, and entoconid.

This comparison is not intended to suggest that the arctocyonids were ancestral

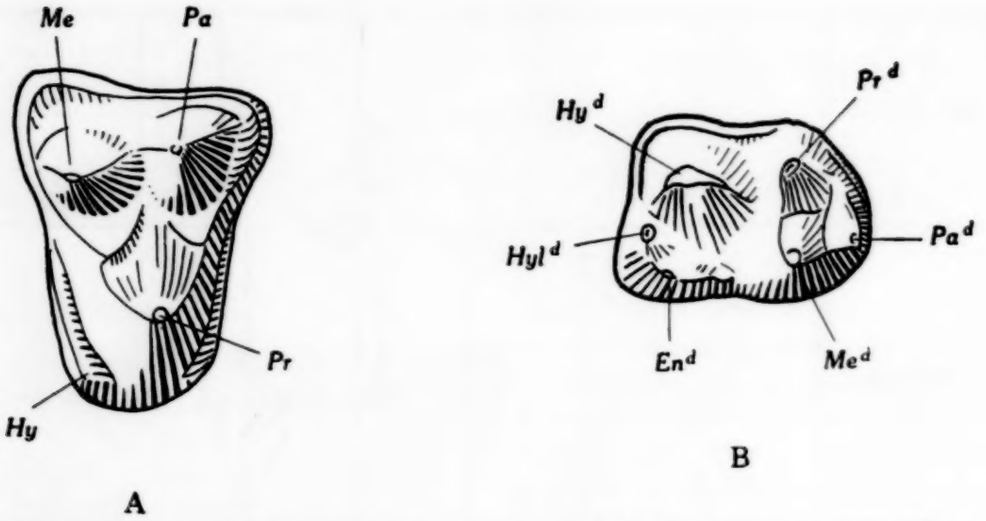

FIG. 2. Molars of the arctocyonid *Oxyclaenus cuspidatus* Cope: A, right M^2; B, left M_2. × 4. Based on Matthew, 1937, fig. 4, and casts. Symbols: *Pr*, protocone, *Pa*, paracone, *Me*, metacone, *Hy*, hypocone, Pr^d, protoconid, Pa^d, paraconid, Me^d, metaconid, Hy^d, hypoconid, En^d, entoconid, Hyl^d, hypoconulid.

to the condylarths, but merely to point out that the trends in arctocyonid molar structure are those, to a lesser degree, of the primitive ungulates, and are not such as would lead to the molar pattern of the other creodont groups.

MESONYCHIDAE

Members of this family appear first in the Middle Paleocene fauna and range through to the Upper Eocene. They vary in size from small to medium, but one example, *Andrewsarchus* from the Upper Eocene of Mongolia, is the largest known land carnivore. The molar structure of mesonychids (fig. 4) is peculiar, and shows a progressive modification towards a premolar-like structure. The upper molars are triangular, with the two outer cusps forming the main part, and the protocone at the end of a narrow internal spur. There is no trace of hypocone. The lower molars are narrow, and have the cusps arranged in a fore-and-aft row. The large middle cone is made up of protoconid and metaconid in the earlier forms, and of a single cusp, presumably protoconid, in the later examples. The paraconid and hypoconid are conspicuous, but much lower than the protoconid. There is no entoconid.

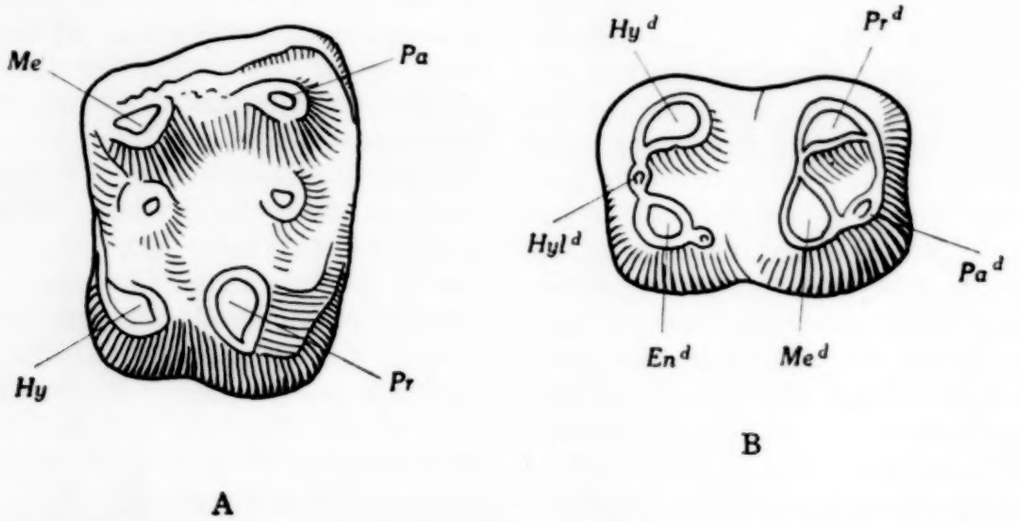

FIG. 3. Molars of the condylarth *Tetraclaenodon puercensis* (Cope): A, right M^2; B, left M_1. × 4. Based on various figures in Matthew, 1937. Symbols as in fig. 2.

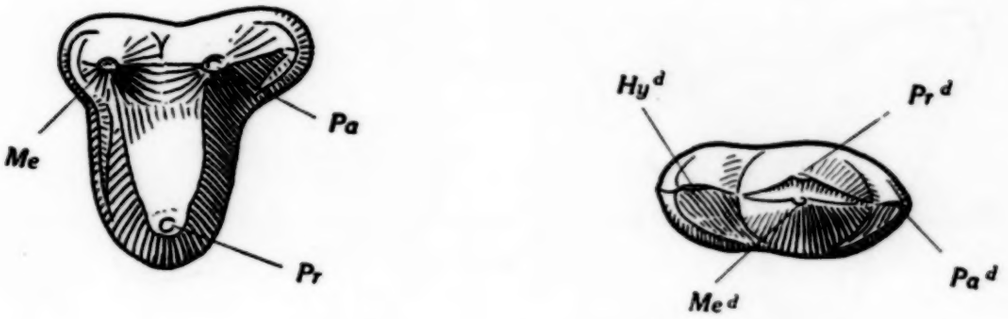

FIG. 4. Molars of the mesonychid *Dissacus navajovius* (Cope): A, right M^2; B, left M_2. ×2. Based on Matthew (1937, fig. 16), Cope (1884, pl. 25c), and specimens. Symbols as in fig. 2.

The characteristic features of the mesonychid molars, especially the retention of the paraconid and the early suppression of the metaconid, make it very unlikely that this family could have been derived from the Arctocyonidae. Compared with other creodont groups, the Mesonychidae show a different approach to the development of a carnivorous dentition, but this could have been derived from teeth such as those of the early miacids. Certainly the trends are widely divergent from those of the Arctocyonidae.

OXYAENIDAE

Members of this family are small to medium-sized forms, appearing in the Upper Paleocene and continuing to the Upper Eocene. In the upper molars (fig. 5A) the crown is triangular, with the external cusps forming a shearing ridge, and the protocone located at the inner end of a narrow spur. M^1, the largest of the upper shearing teeth, is regarded as the carnassial. M^2 is narrow anteroposteriorly and M^3 is reduced or absent. Of the lower molars, M_2 is the carnassial. The trigonid is larger than the talonid, and the paraconid is prominent and projects forward. There is thus much more resemblance to the molars of fissipeds than to those of arctocyonids.

HYAENODONTIDAE

Although this family is usually grouped with the Oxyaenidae in the Pseudocreodi or Oxyaenoidea, it is probable that it represents a parallel, mainly old-world derivative from the early miacids. The oldest known hyaenodonts are from the Lower Eocene, and the family is well represented in the Oligocene. A few genera survived to later times, the last as far as known being from the Lower Pliocene of Pakistan. Most hyaenodontids were of medium size, but there were some small forms and at least one very large one. The molar pattern is similar to that of the oxyaenids, with the outer shearing crest well developed in the upper molars and retention of a prominent paraconid in the lower molars. The largest shearing tooth in the upper dentition is M^2 and in the lower dentition, M_3.

MIACIDAE

In this family the carnassials are P^4 and M_1, as in typical fissipeds, and for this and other reasons the miacids are regarded as the ancestral stock of the Fissipeda. Matthew retained the Miacidae in the Creodonta as the Eucreodi, whereas Simpson removed the group to the Fissipeda as the superfamily Miacoidea. Miacids are known as early as the Middle Paleocene, and persist to the Upper Eocene, above which their place is taken by the canids and other modern fissiped families. In typical miacids the carnassials are not as well developed as in oxyaenids and hyaenodontids. In the upper molars (fig. 6A) the outer cusps are only moderately

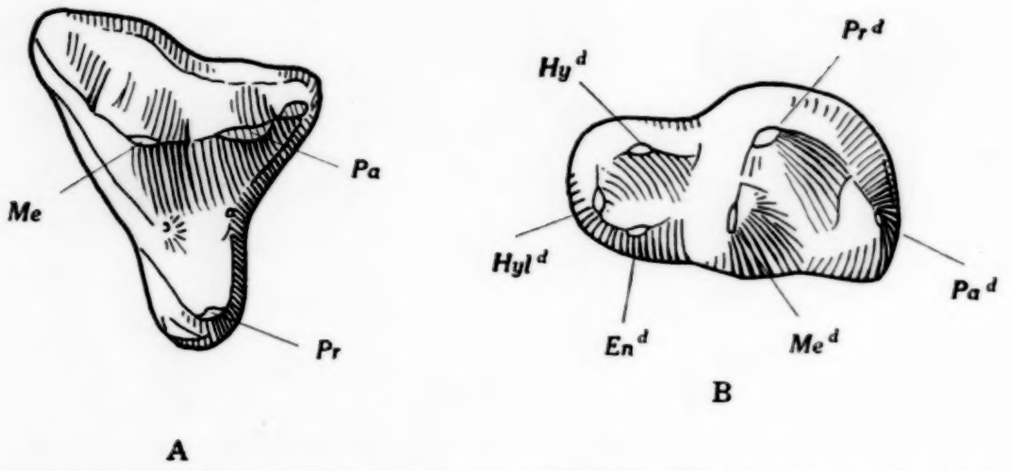

FIG. 5. Molars of the genus *Oxyaena:* A, right M^1 of *O. gulo* Matthew; B, left M_1 of *O. forcipata* Cope. × 2. Based on Matthew (1915, figs. 47, 46).

confluent, and the protocone shelf is relatively broad, with persistent intermediate cuspules. The essential pattern, however, is the same as in the Oxyaenidae and Hyaenodontidae, and the common ancestor of the three families was probably an animal with miacid-like molars. The lower molars of miacids have a large trigonid, with prominent, forward-projecting paraconid. It did not require much modification to transform the miacid dentition into that of the early canids or felids.

CONCLUSIONS

Evidence has been presented to show that from the earliest-known examples the Arctocyonidae show trends in the development of molar structure that are divergent from those seen in the Mesonychidae, Oxyaenidae, Hyaenodontidae and Miacidae. The arctocyonid trends are, in an incipient way, like those of the condylarths, and illustrate the earliest stage in the development of the quadritubercular molars. The other "creodont" families are characterized by the development of dentitions suitable for flesh-eating habits. In the Mesonychidae the molars progressively assume the form of the premolars. In the Oxyaenidae, Hyaenodontidae, and Miacidae one tooth in each row is especially developed for the shearing function. The particular tooth so developed differs in the three families but the structural modification is the same: formation of a paracone-metacone ridge on the upper carnassial and of a para-

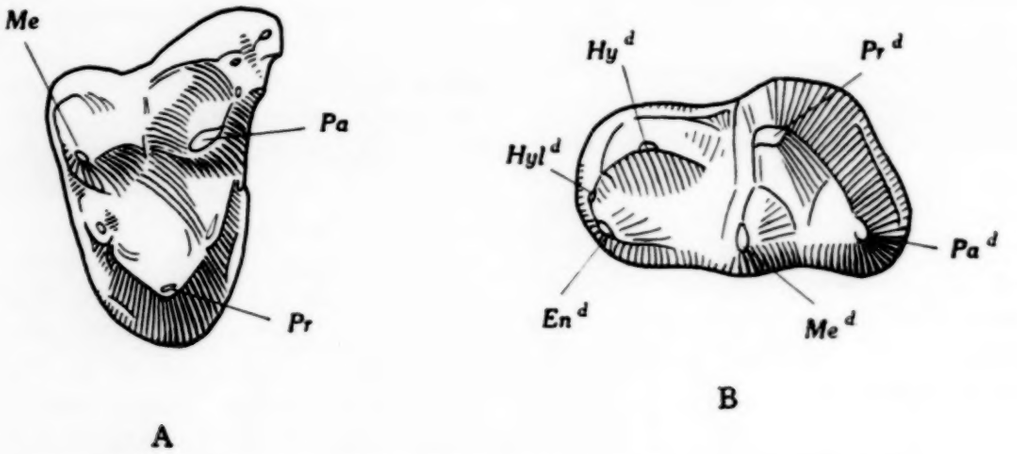

FIG. 6. Molars of the miacid *Didymictis altidens* Cope: A, right M^1; B, left M_1. × 2. Based on Matthew (1915, figs. 16, 17).

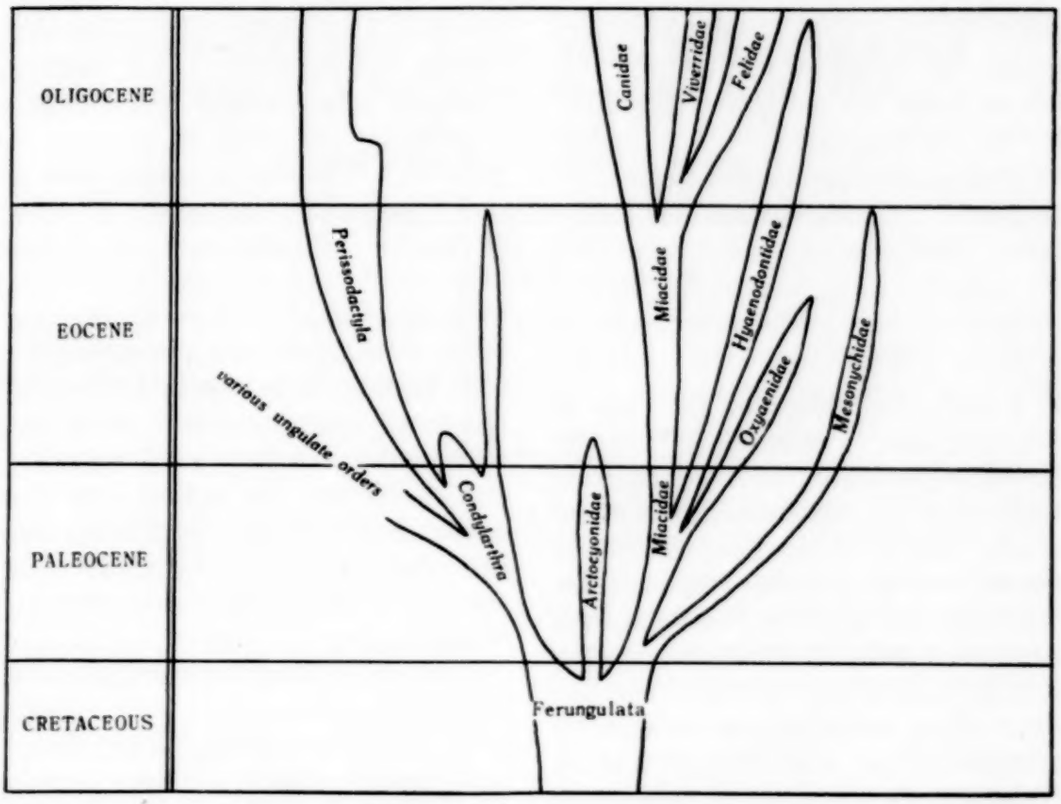

FIG. 7. Phylogenetic tree to illustrate the present conclusions on the relationships of the Arctocyonidae and other "creodont" families.

conid-protoconid-hypoconid ridge on the lower carnassial.

The evolutionary implications of these conclusions are illustrated in figure 7. From the ferungulate group of late Cretaceous time arose two main branches, one leading through the condylarths to the ancestors of many ungulate groups, and the other through primitive miacid-like forms to the various creodonts and fissipeds. The arctocyonids are regarded as a small, intermediate branch of the ferungulates, not much removed from the precondylarth stock. Of the creodont families, the Mesonychidae diverged earliest. Later, from the miacid stem the Oxyaenidae and Hyaenodontidae arose, and developed as more or less parallel lines.

The current classification of the early families of Carnivora obviously would have to be altered considerably to express the above-outlined evolutionary relationships. It is doubtful if they could be expressed fully in the Linnaean system. There does not seem to be sufficient justification for a drastic revision unless it can be part of a major overhaul of mammalian classification.

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EXPERIMENTAL STUDIES OF THE DISTRIBUTION OF GENE FREQUENCIES IN VERY SMALL POPULATIONS OF *DROSOPHILA MELANOGASTER*: I. FORKED [1]

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INTRODUCTION

For quantitative study of the sort of random drift due to inbreeding it is desirable to experiment with segregating genotypes that can be classified without risk of error and with populations that are all of a very small definitely known size.

With respect to the first condition, it is much the most satisfactory if *all* segregating genotypes can be distinguished accurately. This, however, limits severely the number of loci that can be studied. Something can be done where only dominants and recessives can be accurately classified.

Most pairs of alleles with such conspicuous difference in effect as to satisfy the first condition, turn out to be subject to such enormous differences in selection that the accumulation of random deviations, implied by the term random drift, is largely prevented. Selection pressure and the effects of random processes are roughly comparable in magnitude at a given gene frequency if the change in frequency in a generation which the former tends to bring about (Δq) and the variance increment due in a generation to the latter ($\sigma^2_{\delta q}$) are of the same order (Wright, 1931, 1948). Where the ratio ($\Delta q/\sigma^2_{\delta q}$) is as high as 10 in absolute value, there is not much accumulation of random deviations and hence little random drift. The size of experimental populations in studies of the latter, should be small enough to meet this condition. They should be constant for convenient comparison with theory.

The present experiments (all with *Drosophila melanogaster*) were designed to meet these conditions. Three series were performed. About 120 lines were started in each. In the first series, the sex-linked mutation forked (f) competed with its type allele. Four females (1 f/f, 2 f/+, 1 +/+) and 4 males (2 f/0, 2 +/0) were put in each vial. The second series involved the sex-linked semidominant mutation Bar (B) and its type allele. Each initial vial contained 4 B/+ females and 4 males (2 B/0, 2 +/0). The third series was with the autosomal alleles aristapedia, ss^a, and spineless ss; which produce a heterozygote that is close to type. Each initial vial contained 4 ss^a/ss females and 4ss^a/ss males. The alleles were thus equally frequent at the beginning of each experiment.

The cultures were allowed to develop until about 2 to 4 days after the offspring began to emerge. The flies hatched up to this time were discarded (in an evening). The flies which appeared next morning (if enough had emerged) were etherized and from among them 4 females and 4 males were taken at random and served as progenitors of the following generation. The etherized flies were put on a porcelain plate and the first 4 males and the first 4 females that happened to be closest to the

[1] Experimental data by W. E. Kerr, under a fellowship of the Rockefeller Foundation, mathematical analysis by S. Wright. Acknowledgment is made to Dr. Th. Dobzhansky for material and for his hospitality during the conduct of these experiments. Acknowledgment is also made to Dr. J. Crow for material and suggestions. Analysis was aided by a grant from the Wallace C. and Clara A. Abbott Memorial Fund of The University of Chicago.

[2] Editor's Note: This paper is the first of a series of three. The others will be published in successive issues of EVOLUTION.

right end of the plate were the flies taken. It was often, however, necessary to wait to the second and sometimes to the third day to obtain 4 of each sex. This procedure was repeated in every following generation in every line. The first series was carried 16 generations, the second 10 and the third 9 generations. In general, all 8 parents in each culture lived until their progeny started to emerge. In some instances one or more died a few days after the culture was started. No new flies were substituted. Lines were discontinued if fixation was attained. A few were discarded on account of mite infection and other accidents. These latter strains have not been included in the statistics presented here. The present paper will be confined to the experiments with the forked.

Forked

Table 1 shows the most important results. Among the 96 lines with initial gene frequency (.50 f + .50 f⁺), 26 were still unfixed at the end of 16 generations. Wild type had become fixed in 41 and forked in 29. There is here only an insignificant suggestion that forked was at a selective disadvantage. There seems, however, to have been a shift toward increasing disadvantage of forked as the experiment proceeded. In the first 8 generations, wild type became fixed in 17 lines, forked in 23, while in the last 8 generations, wild type became fixed in 24 lines, forked in only 6, a difference with a probability from accidents of sampling of less than .01. Nevertheless it is clear that the selection against forked must have been slight to have permitted as much fixation as occurred against its pressure.

This result is in agreement with those of previous experiments. In Ludwin's (1951) experiments, the initial gene frequency of .50 in cultures which contained on the average about 44 males, 51 females

Table 1. *The amount of fixation of forked and its type allele in 96 lines, each consisting of 4♂'s and 4♀'s in each generation and carried 16 generations unless fixed earlier.* The amount of fixation from generation 4 to 16 is compared with the expected amount at a constant rate of 8.9% per generation.

	Type newly fixed	Not fixed	Forked newly fixed	Total	Observed (fixed)		Calc.		$\frac{(o-c)^2}{c}$
					No. (o)	%	No. (c)	(o-c)	
1	1	94	1	96	2	2.1			
2	0	92	2	94	2	2.1			
3	1	87	4	92	5	5.4			
4	5	79	3	87	8	9.2	7.7	+0.3	.01
5	3	70	6	79	9	11.4	7.0	+2.0	.57
6	1	66	3	70	4	5.7	6.2	−2.2	.78
7	5	59	2	66	7	10.6	5.9	+1.1	.21
8	1	56	2	59	3	5.1	5.3	−2.3	1.00
9	3	52	1	56	4	7.1	5.0	−1.0	.20
10	4	47	1	52	5	9.6	4.6	+0.4	.03
11	5	39	3	47	8	17.0	4.2	+3.8	3.44
12	2	37	0	39	2	5.1	3.5	−1.5	.64
13	3	34	0	37	3	8.1	3.3	−0.3	.03
14	3	30	1	34	4	11.8	3.0	+1.0	.33
15	1	29	0	30	1	3.3	2.7	−1.7	1.07
16	3	26	0	29	3	10.3	2.6	+0.4	.06
1–3	2	273	7	282	9	3.2			
4–8	15	330	16	361	31	8.6	32.1	−1.1	
9–16	24	294	6	324	30	9.3	28.9	+1.1	
4–16	39	624	22	685	61	8.9	61.0		8.37

but with enormous variations, fell to .30 in about 2 or 3 months and was still about the same at 6 months. In experiments by Merrill (1953) in populations that rarely exceeded 100 adults and in many cases were down to less than 10 flies, the frequency of forked similarly fell from .50 at the beginning to .30 by 99 days and averaged .33 in counts from 125 days to 270 days. There was no indication of increasing selection against forked in those experiments. Both authors found evidence of important differences in gene frequency among individual cultures which they attributed to random drift.

From inspection of table 1, it appears that the percentage of fixation (including both that of type and forked) rose to generation 4 but did not change consistently thereafter. The average rate for generations 4 to 16 was 8.91% (standard error 1.09%). Assuming theoretical constancy at this figure, the differences between observed and calculated numbers for unfixed and for newly fixed lines yields $\chi^2 = 8.4$, 12 degrees of freedom, probability .70–.80 of being exceeded by accidents of sampling.

The theoretical rate of fixation for a neutral sex-linked gene after a steady rate has been attained has been given as approximately $(2\ N_M + N_F)/(9\ N_M N_F)$ in which N_M is the effective number of males and N_F that of females. (Wright 1933). This approximation only applies, however, if N_M and N_F are moderately large. The panmictic index P (= 1-F where F is the inbreeding coefficient) measures the amount of heterozygosis relative to that in a random bred stock. The exact recurrence formula derived by the method of path coefficients was given as follows in the paper cited. Primes refer to preceding generations.

$$P = P' - C_1\ (2\ P' - P'') + C_2\ (2\ P'' - P''')$$

where

$$C_1 = (N_F + 1)/(8\ N_F),$$
$$C_2 = (N_M - 1)(N_F - 1)/(8\ N_M\ N_F)$$

In the present case

$$C_1 = 5/32,\ C_2 = 9/128.$$

$$P = .6875\ P' + .296875\ P'' - .0703125\ P'''$$

TABLE 2. *The theoretical values of P (=1−F), P/P′ and the proportional rate of change of P in a population consisting of 4 males and 4 females under sex linked inheritance and no selection*

Generation	P(=1-F)	P/P′	ΔP/P′
0	1		
1	1	1	0
2	1	1	0
3	.914062500	.914062500	.085937
4	.854980469	.935363248	.064637
5	.788848877	.922651341	.077349
6	.731885910	.927789759	.072210
7	.677245259	.925342665	.074657
8	.627418808	.925427759	.073572
9	.580946889	.925931581	.074068
10	.538047138	.926155468	.073845
11	.498260630	.926053861	.073946
12	.461439099	.926099858	.073900
13	.427329066	.926079014	.073921
14	.395744515	.926088456	.073912
15	.366492734	.926084178	.073916
16	.339403833	.926086117	.073914
17	.314316879	.926085236	.073915
18	.291084347	.926085636	.073914
19	.269568980	.926085455	.073915
20	.249643934	.926085538	.0739145

The correlation $(1 - P_0)$ between the gametes that united to produce the foundation females was zero and the correlation $(1 - P_1)$ between the gametes that unite to produce their daughters is also zero. If there were no differential fecundity among the foundation flies there would be no correlation between mating males and females and hence $(1 - P_3) = 0$. From this point, however, the inbreeding coefficient rises. For calculation of P_3, $P'(= P_2)$, $P''(= P_1)$ and $P'''(= P_0)$ are all assigned the value 1. Table 2 shows the values of P, the ratio of successive values P/P' and the percentage change in P per generation $(100\ (P - P')/(P')$ for 20 generations. It may be seen that P/P' oscillates about the value .9260855. This is approximated to 4 places by the 11th generation and to 7 places by the 19th. Values of P beyond this point can thus be calculated to 6 places by the formula $P = .9260855\ P'$.

The rate of decrease of heterozygosis after it reaches stability can also be derived at once by equating P/P', P'/P'' and P''/P''' and expressing in terms of $x = \Delta P/P'$ (Wright 1933).

$$x^3 + x^2\ (2 + 2\ C_1) + x\ (1 + 3\ C_1 - 2\ C_2) + (C_1 - C_2) = 0$$

The solution is .0739145, the limit about which $\Delta P/P'$ oscillates in the successive generations.

The rate of fixation of lines approaches this same value although somewhat more slowly. Actually it has been noted that a practically constant rate 8.91% is attained by F_4. As this is 20.5% larger than the theoretical rate, it is implied that the actual variance due to random processes in each generation was 20.5% greater than expected. As the difference is only 1.5 times its standard error, it is not certainly significant. Taking it at face value, the excess might conceivably be due to fluctuating selection but as there is very little average selection it more probably means that the effective size of population is about 83% of the theoretical value. In an autosomal diploid population the rate of decrease of heterozygosis is $\Delta P/P' = 1/(2N)$ (Wright, 1931). If effective size is defined as $P'/2\ \Delta P$, the theoretical effective size with sex linkage and 4 females and 4 males per generation is 6.7646 while the effective size of the experimental population was 5.61. The difference if real can easily be accounted for if 1 or 2 of the 8 flies fail completely to reproduce in each generation.

The distribution of gene frequencies, during the period of constant rate of fixation, must have practically reached equilibrium of form. The actual distribution was not determined because of the lack of visible distinction between +/+ and +/f. It is of some interest, however, to consider what it must have been. It may suffice to give the distribution for an effective population of 6 and ignore the indicated slight selective differential (Wright, 1931). The standard is considered a population of 6 monoecious diploid individuals with completely random

TABLE 3. *The distribution of unfixed classes after stability of form has been reached in a population in which 2N = 12, no selection; and the distribution in the following generation, including newly fixed classes (each 1/24 of the total unfixed classes of the preceding generation).* The frequency in each of the the unfixed classes is 11/12 of its value in the preceding generation, thus maintaining stability of form.

	Unfixed classes	Following generation
0		.04167
1	.07881	.07224
2	.08985	.08236
3	.09317	.08541
4	.09475	.08685
5	.09554	.08758
6	.09576	.08778
7	.09554	.08758
8	.09475	.08685
9	.09317	.08541
10	.08985	.08236
11	.07881	.07224
12		.04167
	1.00000	1.00000

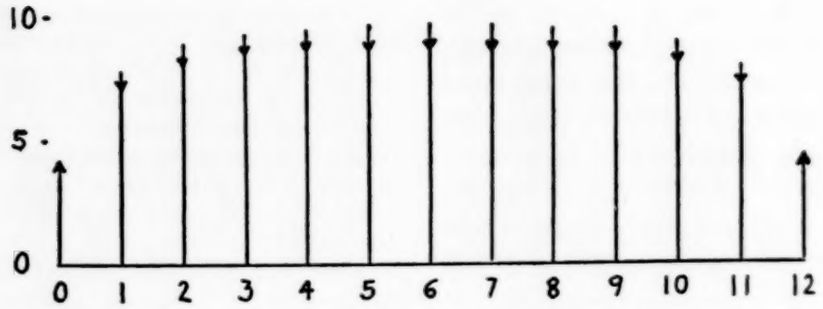

FIGURE 1. The theoretical distribution of gene frequencies (including newly fixed classes) after attainment of stability of form in a population in which $2N = 12$ and selection is absent. Rough estimates for the unfixed classes are given by the ordinates of the curve, $y = 1/12$, unit area (base 12) derived from indefinitely large 2N. The frequencies (1/24) of the newly fixed classes are given exactly by half the terminal ordinates of this curve.

union of gametes, as it has been shown that the distribution is nearly the same for given N_e, irrespective of the system of mating (Wright, 1931).

Let $f(q)$ be the frequency of gene frequency q. The class with this gene frequency contributes to the classes of the next generation according to the expansion of $[(1-q)(f^{+}) + q(f)]^{12} f(q)$ and thus to the class with gene frequency q_1 by

$$\frac{12!}{(2Nq)![2N(1-q)]!} q^{2Nq_1}(1-q)^{2N(1-q_1)} f(q).$$

The total frequency of q is the sum of all contributions from the 11 values of q from 1/12 to 11/12. This must be 11/12 of its value in the preceding generation if there is equilibrium of the form of the distribution. The equation can be solved algebraically as in a number of simple examples given in the reference cited [3] but it is probably simplest in this case to start from a rough approximation and iterate, rating up each generation by 12/11, until stability is reached. The resulting distribution is shown in table 3 and figure 1.

While this solution applies to a situation with a slightly lower rate of fixation (1/24 for each allele) than that observed, the form of the distribution apart from the newly fixed classes is substantially correct for 4 ♀'s, 4 ♂'s (12 representative of the locus) for any effective size of parental population not too remote from 6. Indeed with an indefinitely large population in each generation, the form of the distribution is not very different, being approximately uniform for all gene frequencies (Wright, 1931).

[3] We will note that one of these is incorrect as published, viz., that for irreversible mutation in populations of 3 monoecious individuals (p. 118). The correct percentages are 42.40% for $q_a = 1/6$, 21.07% for $q_a = 2/6$, 15.54% for $q_a = 3/6$, 11.99% for $q_a = 4/6$, 9.00% for $q_a = 5/6$, total 100% for unfixed classes. The rate of fixation of A is 17.05% and of a, 4.53% of the unfixed classes.

TABLE 4. *The history of a line in which an eye color mutation appeared in generation 5 and drifted into fixation in generation 12*

	F_4		F_5		F_6		F_7		F_8		F_9		F_{10}		F_{11}		F_{12}	
	f	w^a	f	w^a	f	w^a	f	w^a	f	w^a	f	w^a	f	w^a	f	w^a	f	w^a
♀ recessive	2	—	1	1	0	0	1	3	0	0	0	2	0	4	0	3	0	4
dominant	2	—	3	3	4	4	3	1	4	4	4	2	4	0	4	1	4	0
♂ recessive	1	—	1	3	0	4	1	0	0	4	0	2	0	3	0	4	0	4
dominant	3	—	3	1	4	0	3	4	4	0	4	2	4	1	4	0	4	0

A situation that arose in one line (No. 109) in this series is interesting. In the 5th generation the flies taken to be parents of the 6th generation were found to be segregating for an eye color found to behave as an allele of white. This gene drifted in frequency until it became fixed in the 12th generation. The frequencies of f and this gene (apparently w^a) were as shown in table 4.

Thus this line had probably become fixed for the type allele of forked by the 9th generation and after the 12 generation for the new eye color mutant.

Discussion

Populations of 4 males and 4 females per generation are so exceedingly small that experiments such as the present may seem to have no implications for evolution in nature. It must be borne in mind, however, that changes in the underlying multifactorial genetic structure of species probably occur so slowly that an appreciable change in a thousand generations must be considered as an explosively rapid process.

Study in the laboratory of the factors that can contribute to such change is practicable only by stepping up the rates by at least one hundred fold. Thus the interaction between a weak selective advantage of one isoallele over another and a slight random drift, due to inbreeding, can be simulated by using alleles with selective differentials of ten percent or more instead of perhaps only one tenth of a percent or even one hundredth of a percent in populations of only one percent or even one tenth of one percent of the size of a typical natural deme.

In the case of forked, the selective differential is clearly much less than ten percent so that the results of the present paper illustrate random drift from inbreeding in an almost pure form. More complicated situations will be considered in the later papers of this series.

Summary

Ninety-six lines of flies (*Drosophila melanogaster*) were started, each from 4 females (1 f/f, 2 f/+, 1 +/+) and 4 males (2 f/0, 2 + /0), and continued to fixation or to the sixteenth generation by random selection of 4 females and 4 males as parents of each new generation. Type (f^+) became fixed in 41 lines, forked (f) in 29 lines, and 26 lines were still unfixed at the end. The amount of selection against forked was thus slight, although there was evidence that it was greater in the later generations than at first.

The rate of fixation (of both alleles combined) reached approximate constancy by the fourth generation at 8.9% per generation. This would imply an effective size of population 83 per cent of that expected under sex linkage with 4 females and 4 males per generation but the reduction is of doubtful significance.

In one line, an eye color mutation, probably apricot, appeared in F_7 and became fixed in F_{12}, three generations after fixation of the type allele of forked.

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NOTES AND COMMENTS

FUNDAMENTALS OF ECOLOGY [1]

RICHARD S. MILLER

In addition to being the first attempt in several years at a concise, introductory treatment of the fundamentals of ecology, this book commends itself to evolutionists as a radical and refreshingly dynamic approach that emphasizes those principles and concepts that apply most to evolutionary problems. The study of group organization, or population and community ecology, is viewed by the author as "the unique part of ecology" that gives it an identity separate from work in other fields. Coupled with a sound, thoughtful treatment of energy relationships as the basis of community organization, this thesis provides a key to the content and organization of the book.

The usual "factor approach," which begins with individual factors and works toward the community level in what is assumed to be a logical development from simple to complex, has been abandoned entirely. Instead the book is divided into three main sections covering (1) the basic principles and concepts that apply to population and community activity generally, (2) freshwater, marine, and terrestrial habitats, their general characteristics and organization; and (3) applied ecology. In other words, the reader is introduced to ecology at the level of complexity he knows, namely the natural community. Individual factors are considered in their specific relations to particular events, but not until a groundwork of governing principles has first been established.

Although the general tone of the book seems often rather elementary, even for the most retarded freshman, some chapters are exceptionally good. Chapter 6 on "Principles and concepts pertaining to organization at the species population level" is without question the most competent and thorough introduction to population ecology that is available to the beginning student in this field. It cannot however, nor is it intended to, replace the definitive treatment of this subject by T. Park in "Principles of animal ecology" (1949), and in general readers will need to refer to this volume for a complete understanding of any particular problem.

In spite of excellent content, well-chosen examples, and a thoroughly reliable digest and outline of most of the basic principles and concepts in the field, this book suffers from inconsistent writing and an unfortunate style of presentation—each topic numbered and discussed under the subheadings *statement, explanation,* and *example.* It is difficult to believe that students and general readers need such a crutch and, certainly, it is not a device that offers serious competition to a well-written text.

GENE DISPERSAL AND THE ETHOLOGY OF THE RHINOCEROTIDAE

G. E. HUTCHINSON AND S. DILLON RIPLEY

Two species of *Rhinoceros* and one of *Dicerorhinus* exist in small numbers in Asia and two species of allied genera in rather larger numbers in Africa. The commonest of the Asiatic species is the Great Indian Rhinoceros *R. unicornis* of which Salim Ali and Ripley in Ali (1949) believe about 300 exist in the territory extending eastward into Assam. Ripley (1952) has brought together the available information on its territorial habits and breeding, which makes a most curious story. The individual animals of either sex live solitarily on definite territories during the greater part of the year. Such territories consist of a small pond around part of which is open grass land. Each territory is marked by a central dunghill and often by small such hills in its peripheral parts. The females apparently come into heat between February and June. The gestation period appears to be nineteen months, followed by a lactation period of several months. The female therefore cannot breed annually. There is evidence derived from captive animals that the male is sexually active for a very short period during the first half of the year. All local observers indicate that periodically the animals leave their territories and wander about over considerable distances. Ripley obtained evidence that this can happen in March, during the middle of the putative breeding season. He supposes

[1] Fundamentals of ecology by Eugene Odum. W. B. Saunders Co., Philadelphia, 1953: 1–384, figs. 1–119, $6.50.

that the wandering permits a female in heat to meet a bull that happens to be sexually active, and that this vague and random method of finding a mate has developed in the absence of environmental control except of the vaguest kind, in an animal whose whole female reproductive cycle from heat to the end of lactation is considerably greater than the annual seasonal cycle. There is a little evidence that in the almost extinct *Dicerorhinus sumatrensis*, the distance of wandering is even greater than in *R. unicornis* (Ansell, 1947). It is said that males and females may mate between July and October. It is obvious that in a population of not more than fifty individuals spread over a wide area from central Burma to southern Malaya random wandering during an ill-defined breeding season is not likely to produce many fertile unions and it is very difficult to see how *D. sumatrensis*, the smallest known living rhinoceros, will be able to survive even if it remains unmolested. Hoogerwerf (1950) reports ten Javan rhinoceros, *R. sondaicus* living in a single reserve in west Java. Nothing is known of this species' breeding behavior.

The African Black Rhinoceros, probably with five valid subspecies, was formerly distributed over the greater part of the thorn scrub and savannah areas of Central Africa; it is still probably the commonest of the five species. The White Rhinoceros, *Ceratotherium simum*, has existed during historic times in two populations which are highly discontinuous and subspecifically distinct, one on the west bank of the Nile in the Sudan and Uganda, the other in the Union of South Africa. Of the southern nominate subspecies, from one to two hundred specimens still survive in the Zululand Game Reserves. In a recent semipopular article Attwell (1948) has given some ethological notes on the rhinoceroses of Zululand that provide an interesting contrast to Ripley's observations. In Zululand the Black and White Rhinoceroses are essentially sympatric, but are ecologically differentiated. The Black feeds on bushes and trees, mainly *Acacia* and *Spirostachys*, while the White eats grasses and small shrubs. The Black appears to live either solitarily or in mated pairs; it is strongly territorial and very static, while the White may form small groups which wander. The curious habit of making a dunghill is exhibited by the White, but the Black, though it deposits its excreta in one place, is careful to spread them as if manuring a garden. It is quite clear that the two African and the Great Indian Rhinoceroses have different specific types of territorial behavior and it seems possible that the smaller almost extinct Asiatic *D. sumatrensis* resembles *R. unicornis* more closely than either African species in this regard.

It is clear, therefore, that within this closely allied assemblage of animals we have very different types of population structure. In *R. unicornis* and perhaps in *D. sumatrensis* the effective breeding population would be, under natural conditions, a very large fraction of the whole population, while in *Diceros bicornis* very small effective breeding populations would be expected. *C. simum* perhaps occupies an intermediate position.

At present the behaviour of *R. unicornis* and still more of *D. sumatrensis* is highly unadaptive. If their populations were larger, as in prehuman times, a rapid distribution of genes throughout the whole population would be expected. In the two African species the opportunities for random fixation of genotypes in local populations must be considerably greater than in the wandering species. It is the purpose of this note to call these conclusions to the attention of palaeontologists and others interested in evolution, as it is just possible that variations in evolutionary rate, dependent on differences in population structure, might be discovered in the different lineages in the family.

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